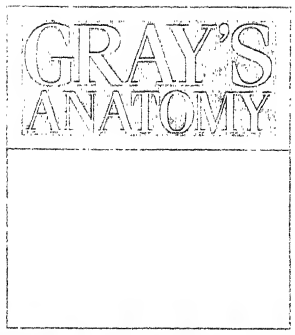


# INTEGUMENTAL SYSTEM: SKIN AND BREASTS

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## OUTLINE OF SECTION

The term *integumental system* is used here to denote the skin and its derivatives: hairs, nails, and sweat and sebaceous glands, the mucocutaneous junctions around the openings of the body orifices, and the breasts. In previous editions of *Gray's* the skin was considered in the Introductory Section with the epithelia and connective tissues, and the breasts were described with the reproductive system. In view of the importance of the skin in human biology, its nature as a composite system of tissues and the common origin of the skin and breasts from an interactive combination of ectoderm and mesoderm, it was felt appropriate to create a separate section to bring these topics together. In this account the external appearance of the integument and its variations in different parts of the body is described first, followed by the microstructure of the epidermis and dermis, and the appendages of skin including the pilosebaceous units comprising hairs, sebaceous and apocrine glands and associated smooth muscle fascicles, and the nails, and sweat glands. Concluding this part of the section, the effects of ageing on skin, and the processes of tissue repair are described briefly; the prenatal development of skin is dealt with in Embryology and Development (p. 294), and details of innervation are in Nervous System (p. 1289) although the development of cutaneous innervation is dealt with in this section on pages 398–399. In the second part of the section the topographic anatomy, microstructure and development of the breasts (female and male) are considered.

## INTRODUCTION

The skin (integument, cutis) is largely ignored by the student in the dissecting room, just being incised, reflected, and cast aside as something which hides more interesting things underneath. Yet, it is that part of the body in which, par excellence, can be demonstrated, at all levels of observation, the relation between structure and function in biological organization. Here also, a number of different body systems come together in synergy to fulfil general overall functions beyond their individual specialized capacities. In recent years, skin has increasingly become a common meeting ground for biologists with a variety of different primary interests, leading to significant cooperative basic and applied research, and the blurring of interdisciplinary boundaries.

The skin covers the entire external surface of the body, including the external auditory meatus, the lateral aspect of the tympanic membrane and the vestibule of the nose. It is continuous with the mucosae of the alimentary, respiratory, and urogenital tracts at their respective orifices, where the specialized skin of mucocutaneous junctions occurs. It also fuses with the conjunctiva at the margins of the eyelids, and with the lining of the lacrimal canaliculi at the lacrimal puncta. Skin forms about 8% of the total body mass, and its surface area varies with height and weight; in an individual of 1.8 m and weighing 90 kg, it is about 2.2 $m^2$. Its thickness ranges from about 1.5–4.0 mm, variations being due to maturation, ageing, and regional specializations.

The skin forms a self-renewing and self-repairing interface between the body and its environment, and is a major site of intercommunication in both directions between the two. Within limits, it forms an effective barrier against microbial invasion, and has properties which can protect against mechanical, chemical, osmotic, thermal and photic damage. It is capable of absorption and excretion, and is selectively and regionally permeable to a variety of chemical substances. It is an important primary site of immunosurveillance against the entry of antigens, and of initiation of the primary immune response. Skin carries out many biochemical synthetic processes (Boyce 1994), including the formation of vitamin D from the precursor 7-dihydrocholesterol under the influence of ultraviolet B (UVB) radiation, synthesis of cytokines and growth factors, etc., and is the target of a variety of hormones. In a sense, it can be regarded as an endocrine organ. These activities can affect the appearance and function of individual skin components, such as the sebaceous glands, the hairs and the pigment-producing cells. Control of body temperature is an important function of skin, being effected mainly by regulation of heat loss from the cutaneous circulation by vascular mechanisms which can rapidly increase or reduce the flow of blood to an extensive surface area exposed to the exterior, assisted by sweating. Skin is involved in sociosexual communication at close quarters and at a distance, and in the case of facial skin, can signal emotional states by means of muscular and vascular responses. It provides individual identification and awareness of personal identity and self-image. Herein lies the concept of 'mind and skin' in the interpretation and treatment of many dermatological disorders which can be cosmetic disasters. It is a major sense organ, richly supplied by nerve terminals and specialized receptors for touch, temperature, pain, mechanical and pleasurable stimuli. The segmental arrangement of the spinal nerves is reflected in the sensory supply of the skin, a *dermatome* being the area supplied by an individual spinal nerve (p. 1289). Knowledge of the spatial distribution of dermatomes is essential for diagnosis of local lesions of nerve roots and of the spinal cord.

Skin has good frictional properties, assisting locomotion and manipulation by its texture. It is elastic, and can be stretched and compressed within limits. The outer surface is covered by various markings, some of them large and conspicuous and others delicate to the point of being microscopic (see Millington & Wilkinson 1983), or only revealed after manipulation or incision of the skin. These are often referred to collectively as *skin lines*, and are considered in greater detail on page 381.

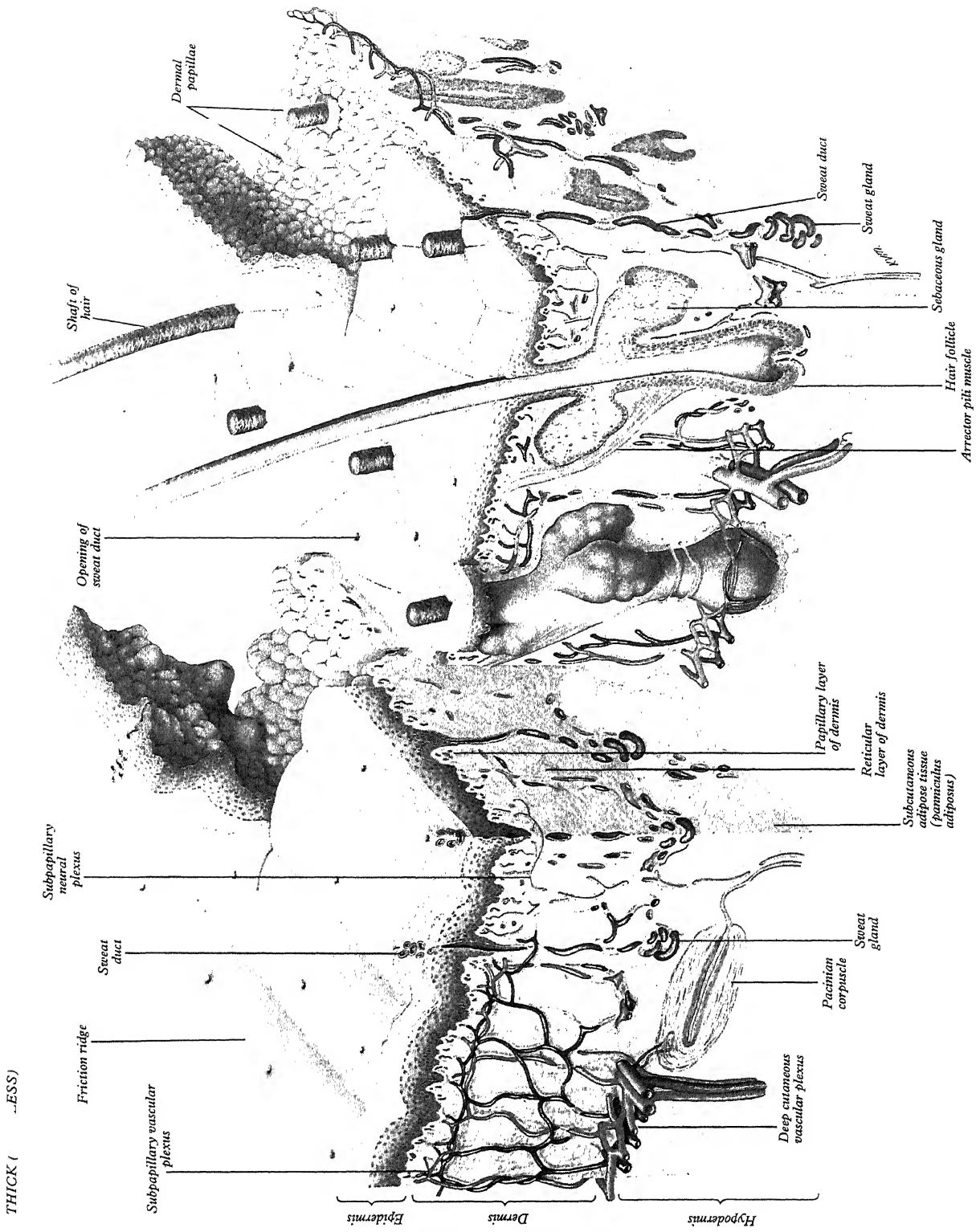
The colour of human skin derives from and varies with the amount of blood (and its degree of oxygenation) in the cutaneous circulation, the thickness of the stratum corneum, and the activity of specialized cells producing the pigment, *melanin*. Melanin has a protective role against ultraviolet radiation, and acts as a scavenger of harmful free radicals produced under this and other circumstances. Racial variations in colour are mainly due to differences in the amount, type and distribution of melanin, and are genetically determined.

In addition to the variations mentioned above, the appearance of skin is also affected by many other factors, for example size and shape and distribution of hairs and their follicles, and of skin glands (sudorific, sebaceous, and apocrine), changes associated with maturation, ageing, metabolic changes, pregnancy, etc. The general state of health is reflected in the appearance and condition of the skin, and the earliest signs of many systemic disorders may be observed by inspecting it. Examination of the skin, therefore, is of importance in the diagnosis of much more than purely skin diseases. Biopsy of fetal skin at amniocentesis is becoming increasingly important for prenatal diagnosis of genetically determined diseases, and can involve the dermatologist in genetic counselling.

For reviews of aspects of structure and function of skin see: Millington and Wilkinson (1983), Thody and Friedmann (1986), Fitzpatrick et al (1987), Champion et al (1991), Goldsmith (1991), and special issues of the current dermatological journals. For Atlases see Breathnach (1971), and Montagna et al (1992). For special techniques related to skin, see Skerrow and Skerrow (1985).

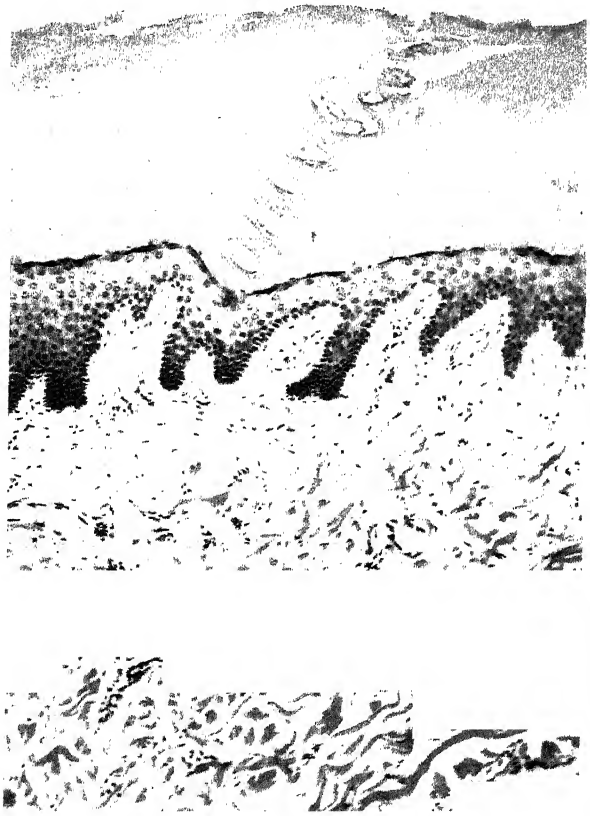
## TYPES OF SKIN

Although skin over the entire body is fundamentally of similar structure, there are many local variations in thickness, mechanical strength, softness, flexibility, degree of keratinization, sizes and numbers of hairs, frequency and types of glands, pigmentation, vascularity, innervation and other features. However, it is useful to distinguish between two major classes of skin which cover large areas of the body, but show important differences of detailed structure and functional properties; these are, *thin, hairy (hirsute) skin*, which covers the greater part of the body, and *thick, hairless (glabrous)*




5.1 Schema showing the organization of skin, comparing the structures present in thick, hairless (plantar and palmar) skin and thin, hirsute skin. The epidermis has been partly peeled back in this picture, to show interdigitating dermal and epidermal papillae. For details of the innervation (yellow), see p. 962.

5.2 Vertical section through the epidermis of the scalp between hair follicles. Note the reduced keratinized layer (compare with 5.3. Haematoxylin and eosin. Magnification x 200.



5.3 Low-power light micrograph of a vertical section through skin from the sole of the human foot: Mallory's triple stain. The finer collagen fibres of the papillary and reticular layers are stained blue and the coarser collagen of the reticular layer stains red. The epidermis is differentiated into the stratum corneum and stratum lucidum above (red-brown) and the strata basale, spinosum and granulosum, which stain blue-grey. A spiral duct from a sweat gland is also seen in vertical section. The base of the epidermis is irregularly ridged. Magnification x 150.

*skin*, forming the surfaces of the palms of the hands, soles of the feet, and flexor surfaces of the digits (5.1–3). These two classes differ in their surface markings, thick skin having complex patterns of friction ridges absent elsewhere on the body. As implied by their names, they also differ in the thickness of both epidermal and dermal components, and in the presence of hairs with attendant sebaceous glands and arrector pili muscles (*pilosebaceous units*). These dissimilarities reflect their distinctive functions. Thick hairless skin forms frictional surfaces for manipulation and locomotion, and requires extra strength for this purpose. It also possesses numerous sweat glands for cooling during sustained activity, and dense clusters of sensitive sensory endings with a high degree of spatial discrimination, unimpeded by the presence of hairs. Thin hairy skin is responsible for the general cutaneous functions over the remainder of the body.

Minor, specialized areas of skin also have distinctive features which do not fall into either of the major categories. The *mucocutaneous junctions* of the lips, outer rim of the anal canal, and urethral opening each have a characteristic histology: for example, the lips have a delicate epidermis lacking glands or hairs, as also do the coverings of the glans penis and glans clitoridis. Some of these regional differences are responsible for local differences in the *microclimate* of the skin surface, and in the bacterial and fungal flora that inhabits it.

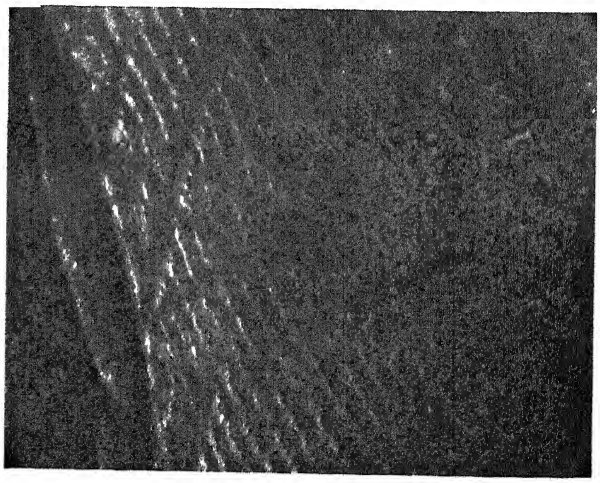
## SKIN LINES

The surface of the skin and its deeper structures show various linear markings. Over 35 different names, many of them synonyms, have been applied to such lines, relating to various systems of grooves, raised areas, preferred directions of stretching, lines of nervous occurrence and spread of infection. Some of these are clearly evident in intact skin, others only appear after some sort of intervention, for example pinching, while the actual existence of others is debatable.

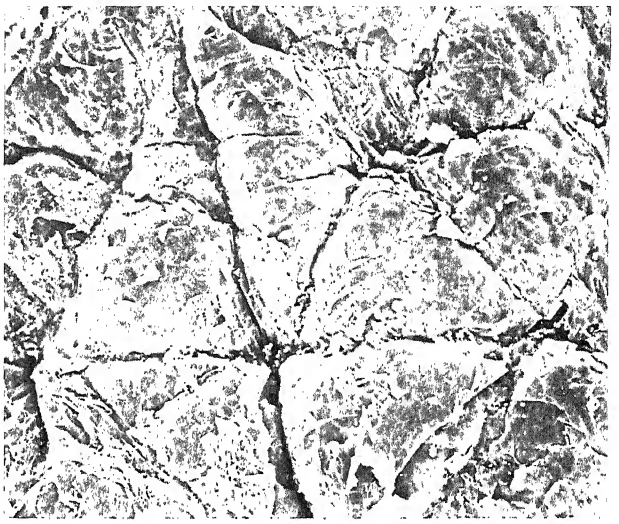
### Externally visible skin lines

Externally visible skin lines are related to various patterns of epidermal creasing, ridge formation, scarring and pigmentation.

**Surface pattern lines, tension lines, skin creases** (5.4–6). A simple lattice pattern of lines occurs on all major areas of the body other than the thick skin of volar and plantar surfaces. The lattice pattern typically consists of polygons (generally parallelograms) formed by relatively deep primary creases visible to the naked eye, irregularly divided by finer secondary creases into triangular areas. These, in turn, are further subdivided by tertiary creases limited to the stratum corneum of the epidermis, and, finally, at the microscopic level, by quaternary lines which are simply the outlines of individual corneocytes (Hashimoto 1974; Millington & Wilkinson 1983). Apart from the quaternary lines, all the others increase the surface area of



5.4 Low-power light micrograph of hairless skin, in surface view, from the palm of the hand showing epidermal friction (papillary) ridges and larger flexure lines (left). Magnification x 6.



5.5 A light micrograph similar to that shown in 5.4 but taken from hairy skin on the extensor aspect of the forearm; note the pattern of surface grooves (tension lines) and hairs. The oblique direction of the emerging hair shafts points away from the pre-axial border of the limb. Magnification × 6.

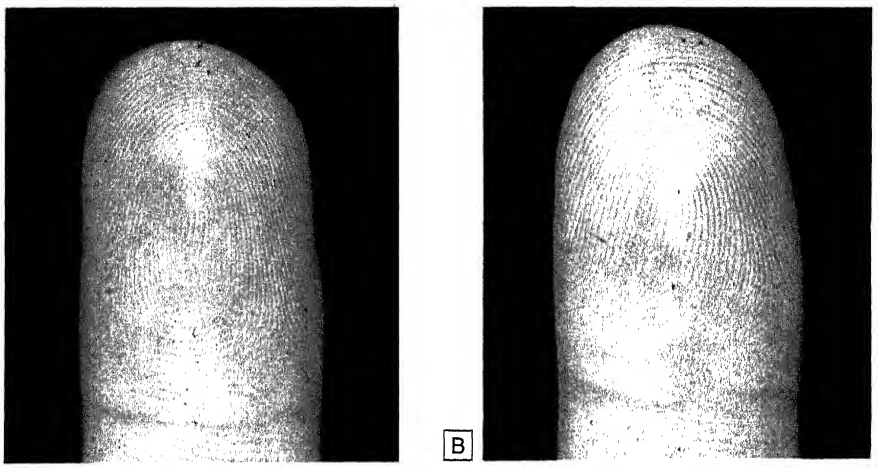
5.6 Scanning electron micrograph of the surface of thin skin (human: dorsum of thorax), showing the interlacing network of fine creases and predominantly triangular areas between them. Magnification × 400.

the skin, permitting considerable stretching and recoil and distributing stresses more evenly. Details of the pattern vary according to the region of the body; for example, on the cheek the primary creases radiate from the hair follicles, on the scalp they form hexagons, while on the calf and thigh they form parallelograms whose longer sides are inclined at about 30–40° to the vertical. There is a relationship between type of pattern and local skin extensibility (Fergus & Barbenel 1981).

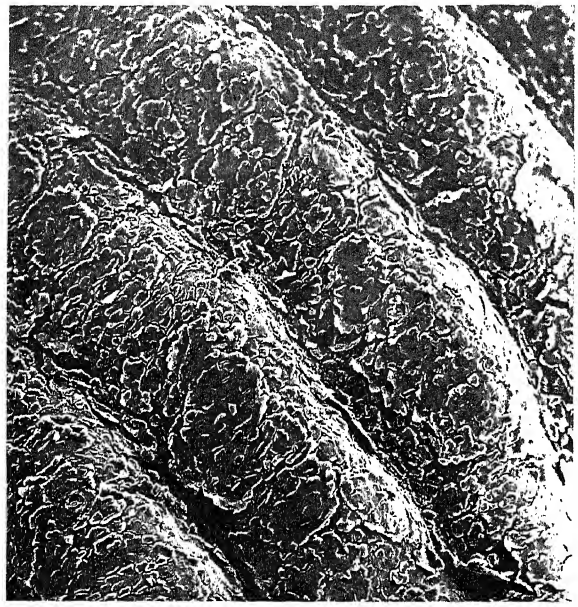
**Wrinkle lines.** These are caused by contraction of underlying muscles and are usually disposed perpendicular to their axis of shortening. On the face they are known as *lines of expression*, and with progressive loss of skin elasticity due to ageing, they become permanent. *Occupational lines* are creases produced by repeated muscular contractions associated with particular trades or skills. *Contour lines* are lines of division at junctions of body planes, for example the cheek with the nose, and *lines of dependency* are produced by the effect of gravity on loose skin or fatty tissue in particular situations, for example the creases associated with the 'turkey-gobbler' fold beneath the chin of the aged.

**Flexure (joint) lines.** These are major markings found in the vicinity of synovial joints, where the skin is attached strongly to the underlying deep fascia (5.4). They are conspicuous on the flexor surfaces of the palms, soles, and digits, and in combination with associated skin folds facilitate movement. The skin lines do not necessarily coincide with the associated underlying joint line. For example, the flexure lines demarcating the extended fingers from the palm lie approximately half an inch distal to the metacarpophalangeal joints, the positions of which are more closely related to the distal palmar crease ('heart-line'). The patterns of flexure lines on the palms and soles may vary and are to some extent genetically determined; the belief that the palmar pattern can reveal personality traits or the future of the individual underlies the practice of palmistry. In Down syndrome, the distal and middle palmar creases tend to be united into a prominent single transverse one, a sign which is of some diagnostic importance.

**Papillary ridges (friction ridges).** These are confined to the palms and soles and the flexor surfaces of the digits, where they form narrow parallel and often curved arrays separated by narrow furrows (5.7, 8). Along the summit of each ridge the apertures of sweat ducts open at regular intervals. The epidermal ridges correspond to an underlying interlocking pattern of dermal papillae, an arrangement which helps to anchor the two components firmly together. The pattern of dermal papillae determines the early development of the epidermal ridges, the arrangement of which is stable throughout life, unique to the individual, and, therefore, significant as a means of identification. The ridge pattern can be affected by certain abnor-




5.7 Photographs of the palmar aspect of a terminal phalanx in two different individuals to show the major types of pattern of the fingerprint ridges. The pattern in (A) is commonly termed a whorl; (B) is composed of loops. Note interphalangeal flexure lines.

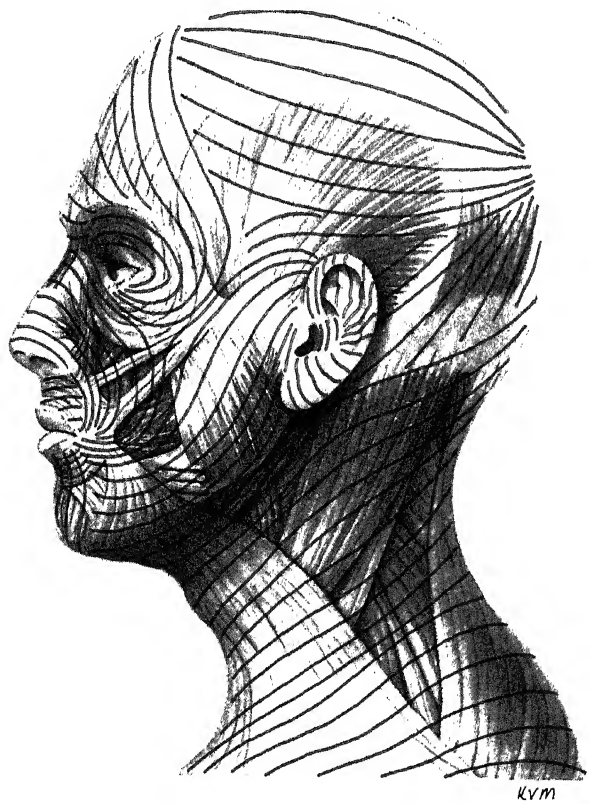


**5.8** Scanning electron micrograph of the surface of thick hairless skin from the volar surface of a human digit, showing friction ridges along which open lines of sweat ducts. Magnification ×400. (Provided by Caroline Wigley, Department of Anatomy and Cell Biology, UMDS, London.)

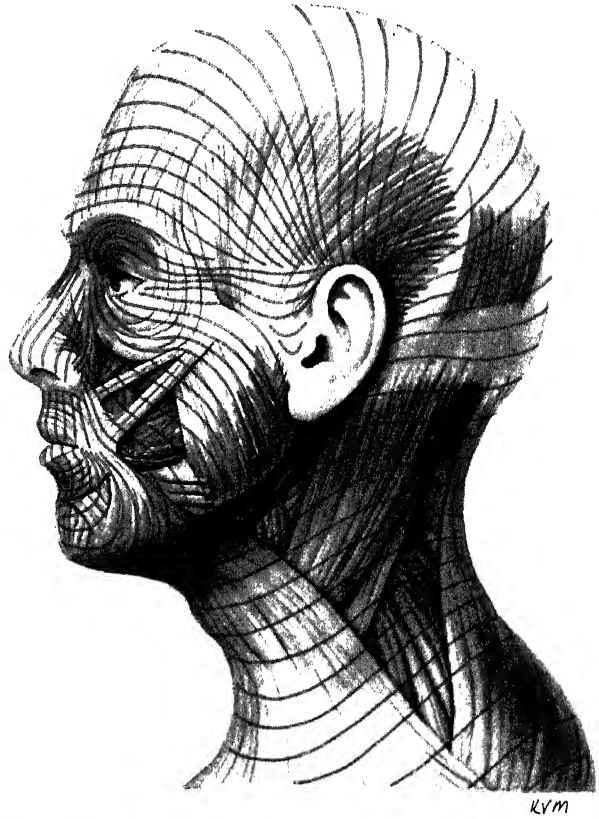
malities of early development, including genetic disorders such as Down syndrome, and skeletal malformations such as polydactyly (Thompson & Bandler 1973). Absence of epidermal ridges occurs in less than 1% of people. Functionally, epidermal ridges increase the gripping ability of hands and feet, preventing slipping, and because of the great density of tactile nerve endings beneath them they are also important sensory structures.

The analysis of ridge patterns by studying prints of them is known as *dermatoglyphics*. It has considerable forensic importance, and there are also complicated mathematical ramifications of this subject which are beyond the scope of this account (see Cummins 1926, 1964; Cummins & Midlo 1961; Penrose & Loesch 1969; Schaumann & Alter 1976). Measurable parameters include the frequency of ridges in particular patterns and the disposition of *tri-radii*, junctional areas where three sets of parallel ridges meet. Ridge configurations may differ on the terminal segments of an individual's fingers and can be separated into three major types (**5.7**): *arches* (5%), *loops* (70%), and *whorls* (25%), arches having no tri-radii, loops one, and whorls two or more. Arches may be simple or tented, loops may have a tri-radius towards the ulnar or radial side of the hand, and whorls may be symmetrical, spiral or double loop. Whorl finger patterns are more common on the right hand, and males generally have more whorls and fewer arches than females, in whom the ridges are relatively narrower. The frequency of individual patterns varies with particular fingers. Ridges within the patterns may branch or join, or may be discontinuous. Similar considerations apply to the toes. In any configuration the number of ridges may vary, the *ridge-count* being given by counting the total intersected when a line is drawn from the central point of a pattern to its nearest tri-radius. The variable features provide an astronomical number of possible combinations, so that each individual is almost certain to have a unique set of patterns.

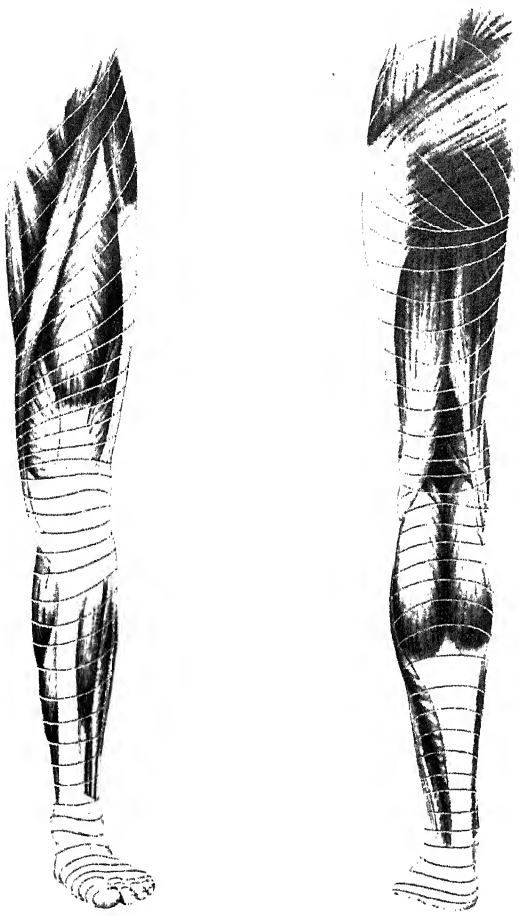
Tri-radii also occur on the palms and soles, including the bases of each digit except the thumb; a characteristic one (the axial tri-radius) is also present on the proximal edge of the hand in the midline above the flexor retinaculum. On the palm there are six areas—interdigitals I–IV, hypothenar and thenar. The precise positions, numbers and ridge-counts associated with the tri-radii have an inherited basis and in general the genetics are multifactorial and highly complex. However, the total ridge-count of all 10 digits of the hand appears to have a simpler inheritance.




**5.9A.** Distribution of Langer's cleavage lines on the face. For further details see text.




**5.9B.** Distribution of the lines of Kraissl's (relaxed skin tension lines) on the face. It has been reported that incisions made along these lines heal with minimum scarring (see text).



5.10 Lines of Kraissl associated with the anterior (left) and posterior (right) aspects of the lower limb.

**Intrinsic scarring.** If the mechanical demands placed on the skin are greater than the skin creases and the dermis can accommodate, the lateral cohesion of dermal collagen fibres is disrupted, with associated haemorrhage and cellular reaction, and eventually, formation of poorly vascularized scar tissue. Such changes can be termed *intrinsic*, to distinguish them from scars formed by external wounding (see p. 412). Sites of dermal rupture are visible externally as lines, *striae*, or stretch marks, which are initially pink in colour, but later widen and become a vivid purple or red (*striae rubrae*), and eventually fade, becoming paler than the surrounding intact skin (*striae albae*). They develop on the anterior abdominal wall of some women in pregnancy when they are termed *striae gravidarum*, associated with stretching due to the growing fetus, and in other conditions involving excessive stretching of the skin such as hypertrophy of muscle in weight-lifters and body-builders, gross obesity and rapidly growing tumours. They tend to follow Langer's lines (see below). There is thought to be a hereditary factor involved in the tendency to develop striae, and they are commoner in conditions of increased adrenal cortical activity and of excess glucocorticoids, and may be side-effects of therapeutic administration of local or systemic steroid therapy (Geschwandtner 1973; Moretti & Rebora 1976). Epidermal 'oedema' and damage to melanocytes occurs in striae rubrae; in striae albae, though melanocytes are present, they are almost totally inactive (Breathnach 1976).

**Pigmentation.** Variation in pigmentation can also produce externally visible lines, such as *Voigt* and *Futcher lines* on the surface of the skin. Voigt lines mark differences in pigmentation between the darker extensor and paler flexor surfaces of the arms, occurring along the anterior axial lines, extending from the sternum to the wrist. They are more common in highly pigmented races (McLaurin 1988).

### Lines detectable after manipulation or incision

**Lines of Langer and Kraissl** (5.9, 10). Skin is normally under tension, the direction in which this is greatest varying regionally. Langer (1861) illustrated patterns of parallel cleavage lines which indicate the direction of elastic tension of skin in particular areas, and it has long been suggested that surgical incisions should be made parallel with them to minimize postoperative scarring. Unfortunately, the lines mapped out by Langer on cadavers do not always coincide with the lines of greatest tension in the living, and Kraissl's (1951) lines which often coincide with wrinkle lines are probably more appropriate lines for surgical incision. Lines of greatest tension similar to those of Kraissl have been termed 'relaxed skin tension lines' by Borges and Alexander (1962).

**Blaschko lines.** These refer to the way in which patterns of naevi and related dermatological pathologies are distributed or develop along certain preferred cutaneous pathways (Blaschko 1901; Rieger et al 1994). They do not appear to correspond to vascular or neural elements of the skin, and may be related to earlier developmental boundaries of a 'mosaic' nature. For more on physical properties, see Marks et al (1988).

## SKIN

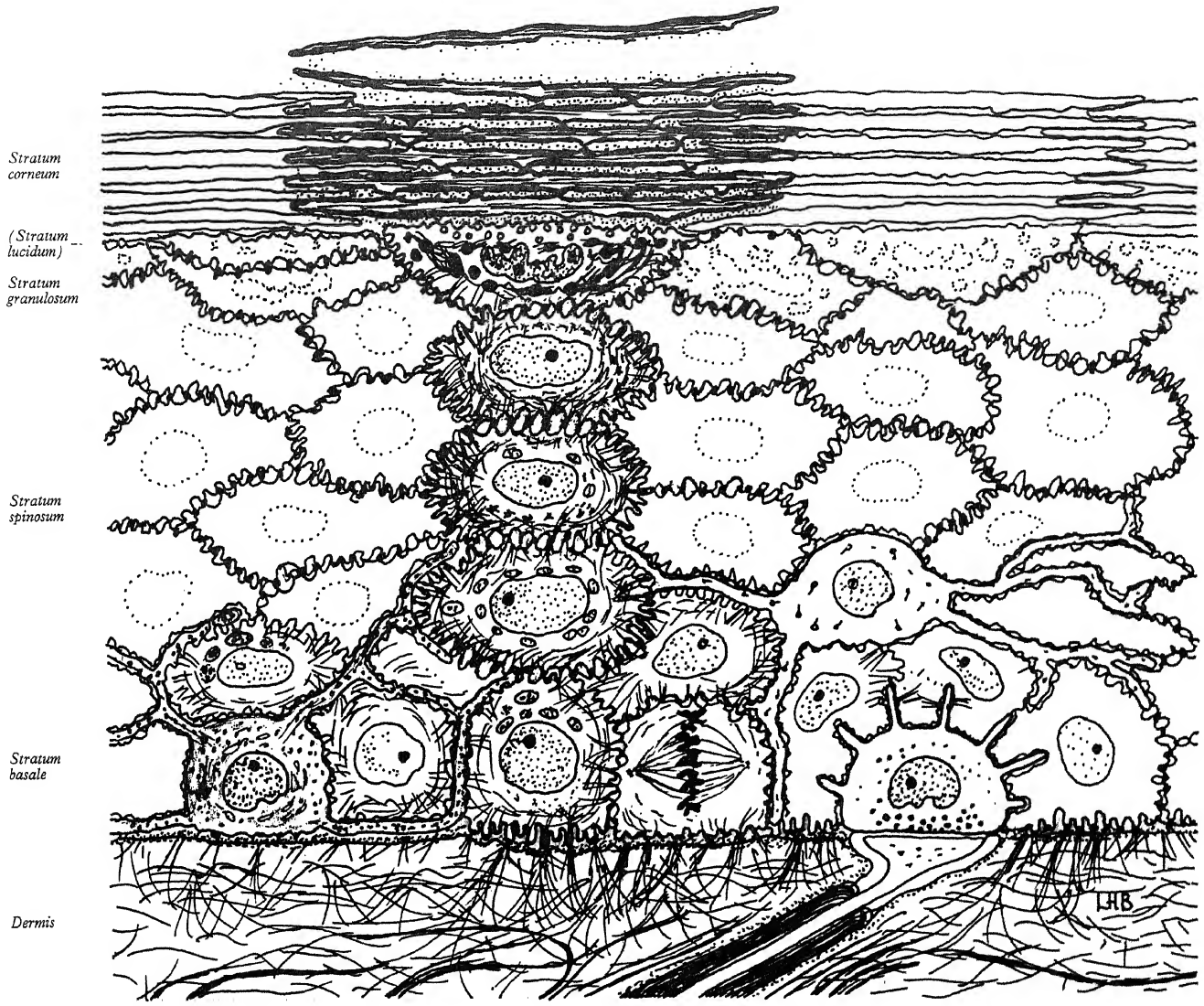
## EPIDERMIS

The epidermis (5.1–3, 11) is a compound tissue consisting mainly of a continuously self-replacing stratified keratinized squamous epithelium, the principal cells of which are called *keratinocytes*. Other cellular elements of different developmental origin within the mature epidermis include *melanocytes* or pigment-forming cells from the embryonic neural crest, *Langerhans cells* which are immunocompetent antigen-presenting cells derived from bone marrow, and *lymphocytes*. These disparate cells are collectively known as *non-keratinocytes* or *epidermal immigrants*. Neurally-associated *Merkel cells* are now thought to be modified keratinocytes. Sensory nerve endings are also sparsely present within the epidermis. Each component has an individual primary function, but the fact of their intimate spatial association and of functional interactions between them has led to the concept of epidermal symbionts (see p. 395). In routine sections stained with haematoxylin and eosin the non-keratinocytes and Merkel cells are poorly distinguishable, appearing as 'clear cells', due to shrinkage with a resulting clear space around them, and/or the absence of keratin filaments from the cytoplasm. However, they can be individually visualized by special light microscopic techniques or electron microscopy. The population of keratinocytes undergoes continuous renewal throughout life, a mitotic layer of cells at the base replacing those shed at the surface. As they move away from the base of the epidermis, the keratinocytes undergo progressive changes in shape and content, eventually transforming from polygonal living cells to non-viable flattened *squames* full of the protein *keratin*, a process known as keratinization.

It is usual to divide the epidermis into a number of strata from deep to superficial as follows: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum (where present) and stratum corneum. The first three of these layers are metabolically active and are often grouped together as the *stratum Malpighii*. They really do not form distinct superimposed layers, and are better thought of as compartments through which cells pass, and change form as they progressively differentiate. The more superficial strata of cells achieving terminal keratinization constitute the *cornified zone*, and here, horizontal, and in some species, also vertical, layering, is evident. *Keratinization* involves not only structural changes in keratinocytes, but also alterations in their relationships with each other and with non-keratinocytes, and chemical changes within the intercellular space. The epidermal appendages (pilosebaceous units, sudoriferous glands and nails) are formed by ingrowth or other modification of the general epidermis, which is often therefore referred to as the *interfollicular epidermis*.

### Keratinocyte strata of the interfollicular epidermis

**Stratum basale.** This includes the deepest layer of cells adjacent to the dermis, and in preparations stained by the periodic acid-Schiff (PAS) reaction or by silver impregnation, appears to rest upon a

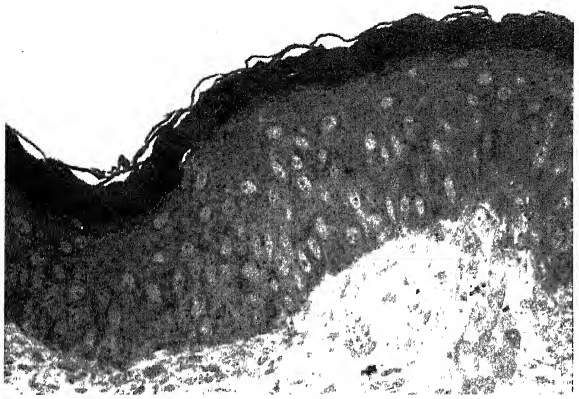



**5.11** Diagram of the main features of the epidermis, including its cell layers and different types of cell, including: keratinocytes (pink); two dendritic varieties, the melanocyte (grey) and Langerhans cell (blue); and a Merkel cell (purple). Also depicted are the dermis (green) and the sensory axon (yellow) associated with the Merkel cell. In this picture the epidermis of thin skin is shown so that the position of the stratum lucidum as it would appear in thick (hairless) skin is only indicated. For clarity only a single column of keratinocytes arising from the mitosis of a basal cell is shown in any structural detail, although desmosomal contacts are not illustrated because of the level of magnification.

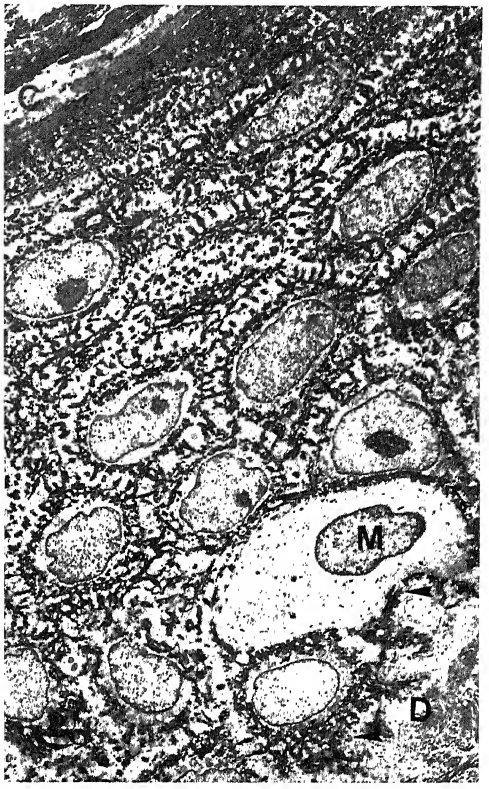
continuous narrow 'basement membrane'. However, electron microscopy has shown that this is not an individual structure, and that the area apparently occupied by it, '*the basement membrane zone*' (BMZ; **5.13**, 39) includes the basal plasma membrane of the cell, a *basal lamina* consisting of lamina lucida and lamina densa, and a dermal *reticular lamina* (see p. 397). This area is also known as the *epidermal–dermal junction* (see later, p. 397). The majority of basal layer cells are columnar to cuboidal in shape, with large, mainly euchromatic nuclei and prominent nucleoli. The cytoplasm contains the common cellular organelles, variable amounts of melanosomes, and, characteristically, many cytoskeletal intermediate filaments including lower molecular-weight keratin filament bundles corresponding to the tonofilaments and tonofibrils of classical light microscopy. The plasma membranes of apposed cells are connected by desmosomes, and the basal plasma membrane has hemidesmosomes distributed along it. Occasionally, spindle-shaped 'dark cells' with more dense cytoplasm, coarser filamentous bundles and heterochromatic nuclei are seen in the basal layer. These have variably been interpreted as 'stem cells', or, more likely, older cells at a premortal stage. Melanocytes, Langerhans cells and (locally in tactile areas) Merkel cells are interspersed among the basal keratinocytes (see p. 394). Merkel cells are connected to keratinocytes by desmosomes, but the other two lack these specialized contacts. *Intraepithelial lymphocytes* are also present in small numbers.

**Stratum spinosum (prickle cell layer).** This contains several layers of more mature keratinocytes packed closely and interdigitating by means of numerous projections and indentations of the cell membranes which are linked by many desmosomes (**5.11–18**), features which provide much tensile strength and coherence to the layer; gap junctions are sporadically present (**5.18A**). When skin is processed for routine histology, the cells tend to shrink away from each other except where joined by desmosomes, so giving them a spiny appearance (hence the name *spinous cells* or *prickle cells* for keratinocytes in this layer, and also the name of the stratum). Internally, spinous cells contain prominent bundles of keratin filaments, arranged concentrically around the moderately euchromatic nucleus, and attached to the dense plaques of desmosomes peripherally (for more on the general structure of desmosomes see p. 27). The cytoplasm contains the common organelles, including some lysosomes and melanosomes, the latter occurring singly or aggregated within membrane-limited organelles (compound melanosomes). Langerhans cells and the occasional associated lymphocyte are the only non-keratinocytes present in the stratum spinosum.

**Stratum granulosum.** In this stratum of three to four layers of



5.12 Section through epidermis and papillary dermis. Note the cellular organization of the epidermis, including the densely staining stratum corneum at the surface. Epoxy resin section stained with basic fuchsin and methyline blue. Magnification ×320. (Provided by Professor R Eady, St John's Dermatology Centre, UMDS, St Thomas' Campus, London.)




5.13 Section of full thickness of epidermis. Note melanocyte (M) in the basal layer, the characteristic 'prickle-cell' appearance of the cells of the stratum spinosum due to the arrangement of their desmosomal contacts, the flattening of the cells of the stratum granulosum, and the squames of the stratum corneum (C). D, dermis, and arrowheads indicate the epidermal–dermal junction. Magnification approx. ×2560.

flattened cells, extensive changes in keratinocyte structure occur (Holbrook 1989). The nuclei become pycnotic and begin to disintegrate, the membranous organelles such as mitochondria, Golgi membranes and ribosomes degenerate, and keratin filament bundles become more compact and associated with, or enmeshed by, stellate or irregular densely staining *keratohyalin granules*. Small round granules (100 by 300 nm) with a lamellar internal structure (*lamellar granules*, *Odland bodies*, *membrane-coating granules*) also appear in the cytoplasm. Keratohyalin granules contain a histidine-rich, sulphur-poor protein (*profilaggrin*) which, when the cell reaches the stratum corneum, becomes modified to *filaggrin* which is thought to provide the interfilamentous matrix and to be concerned with aggregation of the keratin filaments of the corneocyte (see below under 'Epidermal keratinization'. The lamellar granules are concentrated deep to the plasma membrane of the granular cell, with which they fuse, liberating their predominantly lipid contents into the intercellular space not only of this stratum, but also into the space between it and the stratum corneum. They form an important component of the permeability barrier of the epidermis (see below, p. 386). Langerhans cells may occasionally be seen at lower levels of the stratum granulosum.

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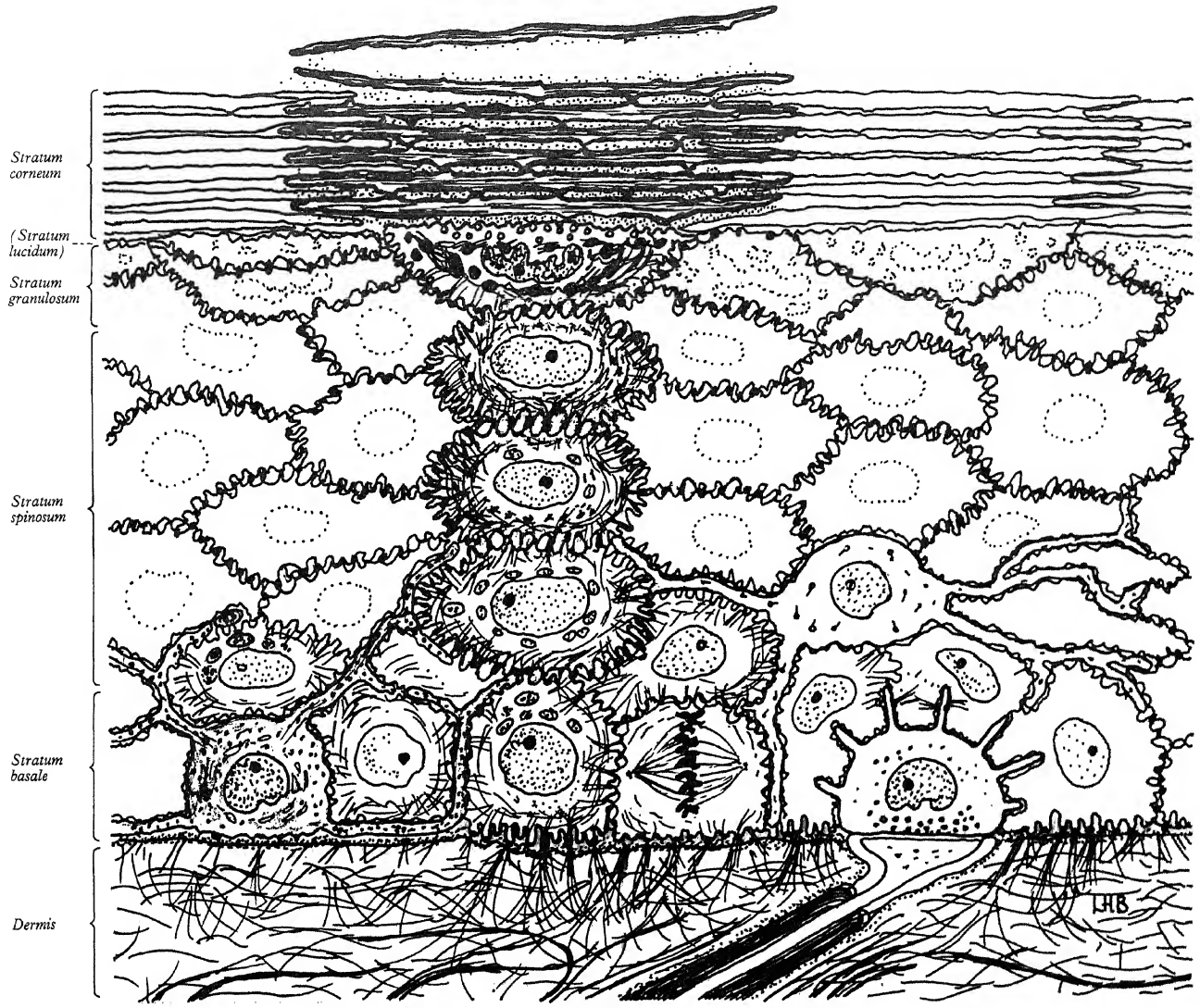
Desquamation of the outer layers of the stratum corneum involves a poorly understood loosening of attachments (desmosomes and intercellular substances) between the cells, probably involving enzyme action, and is normally imperceptible. When excessive, it appears in hairy regions as dandruff, and more massively in certain diseases as peeling, scaling and exfoliation. Langerhans cells are not present in the stratum corneum, and, therefore, are not desquamated.

Though the stratum corneum consists of individual squames, each of which can be consecutively stripped with adhesive tape, it is important to think of it functionally also as an entity which can be separated as a single pliable sheet (Kligman 1964). The thickness of the cornified layer can be influenced by local environmental factors, particularly abrasion, which can lead to a considerable thickening of the whole epidermis including the stratum corneum, so that the soles of the feet become excessively resistant if shoes are dispensed with, and keratinized pads develop in areas of habitual pressure, for example corns from tight shoes, palmar calluses in manual workers, digital calluses in guitar players, etc. Exposure to high levels of sunshine or other stressful agents may also cause general epidermal thickening.

## Epidermal keratinization

Traditionally, epidermal keratinization applied only to the final stages of keratinocyte differentiation and maturation, during which cells are converted into tough cornified squames. However, nowadays

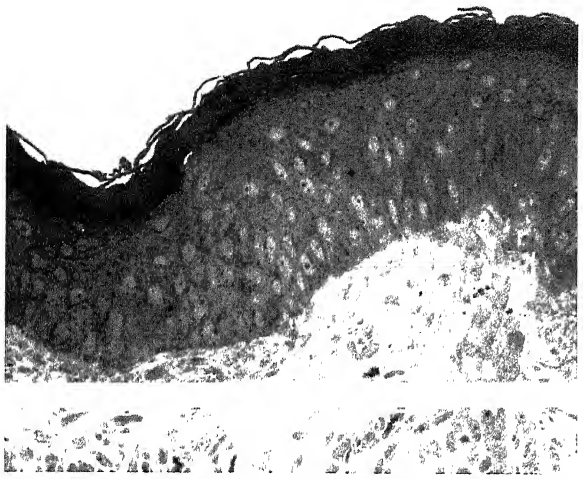



5.11 Diagram of the main features of the epidermis, including its cell layers and different types of cell, including: keratinocytes (pink); two dendritic varieties, the melanocyte (grey) and Langerhans cell (blue); and a Merkel cell (purple). Also depicted are the dermis (green) and the sensory axon (yellow) associated with the Merkel cell. In this picture the epidermis of thin skin is shown so that the position of the stratum lucidum as it would appear in thick (hairless) skin is only indicated. For clarity only a single column of keratinocytes arising from the mitosis of a basal cell is shown in any structural detail, although desmosomal contacts are not illustrated because of the level of magnification.

continuous narrow 'basement membrane'. However, electron microscopy has shown that this is not an individual structure, and that the area apparently occupied by it, '*the basement membrane zone*' (BMZ; 5.13, 39) includes the basal plasma membrane of the cell, a *basal lamina* consisting of lamina lucida and lamina densa, and a dermal *reticular lamina* (see p. 397). This area is also known as the *epidermal–dermal junction* (see later, p. 397). The majority of basal layer cells are columnar to cuboidal in shape, with large, mainly euchromatic nuclei and prominent nucleoli. The cytoplasm contains the common cellular organelles, variable amounts of melanosomes, and, characteristically, many cytoskeletal intermediate filaments including lower molecular-weight keratin filament bundles corresponding to the tonofilaments and tonofibrils of classical light microscopy. The plasma membranes of apposed cells are connected by desmosomes, and the basal plasma membrane has hemidesmosomes distributed along it. Occasionally, spindle-shaped 'dark cells' with more dense cytoplasm, coarser filamentous bundles and heterochromatic nuclei are seen in the basal layer. These have variably been interpreted as 'stem cells', or, more likely, older cells at a premortal stage. Melanocytes, Langerhans cells and (locally in tactile areas) Merkel cells are interspersed among the basal keratinocytes (see p. 394). Merkel cells are connected to keratinocytes by desmosomes, but the other two lack these specialized contacts. *Intraepithelial lymphocytes* are also present in small numbers.

**Stratum spinosum (prickle cell layer).** This contains several layers of more mature keratinocytes packed closely and interdigitating by means of numerous projections and indentations of the cell membranes which are linked by many desmosomes (5.11–18), features which provide much tensile strength and coherence to the layer; gap junctions are sporadically present (5.18A). When skin is processed for routine histology, the cells tend to shrink away from each other except where joined by desmosomes, so giving them a spiny appearance (hence the name *spinous cells* or *prickle cells* for keratinocytes in this layer, and also the name of the stratum). Internally, spinous cells contain prominent bundles of keratin filaments, arranged concentrically around the moderately euchromatic nucleus, and attached to the dense plaques of desmosomes peripherally (for more on the general structure of desmosomes see p. 27). The cytoplasm contains the common organelles, including some lysosomes and melanosomes, the latter occurring singly or aggregated within membrane-limited organelles (compound melanosomes). Langerhans cells and the occasional associated lymphocyte are the only non-keratinocytes present in the stratum spinosum.

**Stratum granulosum.** In this stratum of three to four layers of



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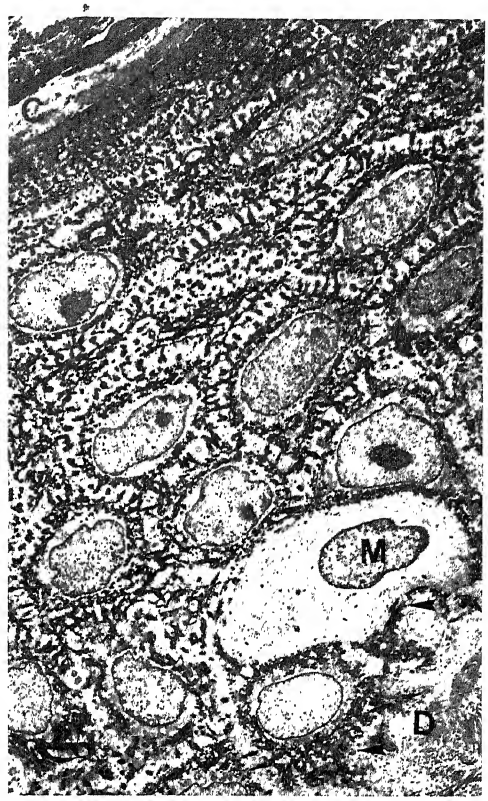


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barrier'). The interior of the corneocyte is devoid of nucleus and membranous organelles, consisting solely of a dense array of keratin filaments embedded in an interfilamentous matrix (5.15) partly composed of filaggrin derived from keratohyalin granules. In stained thin sections different 'keratin patterns' of arrangement of filaments and matrix have been described, which seem to vary with technique of processing as well as with the processor. Further consideration is given to corneocyte structure in the section dealing with the overall process of keratinization (see below).

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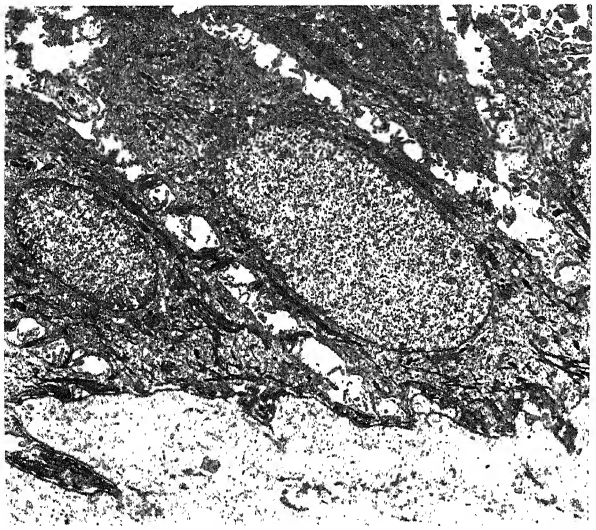
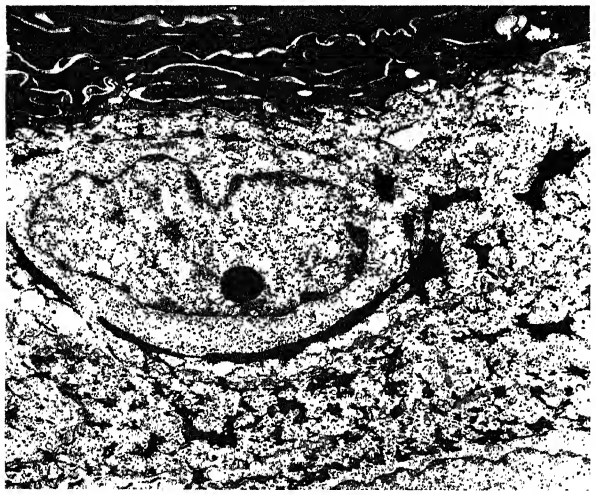


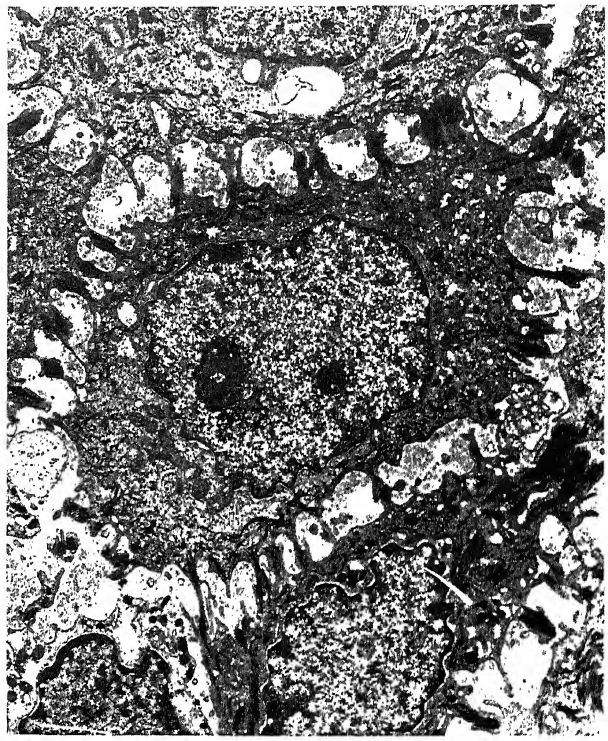
5.14A. Electron micrograph of human epidermis showing a section through the stratum basale where it abuts the basal lamina and papillary layer of the dermis (below). Note the presence of numerous melanin granules in the epidermal cells. Magnification × 7000.



5.14C. Electron micrograph of human skin in vertical section. This shows the transition between the stratum spinosum (below), stratum granulosum (middle) and stratum corneum (the dark laminae above). The cytokeratin bundles of the keratinocytes, below, become denser and more compact in the stratum granulosum; finally the cells flatten, becoming scale-like and electron dense. Magnification × 8000.

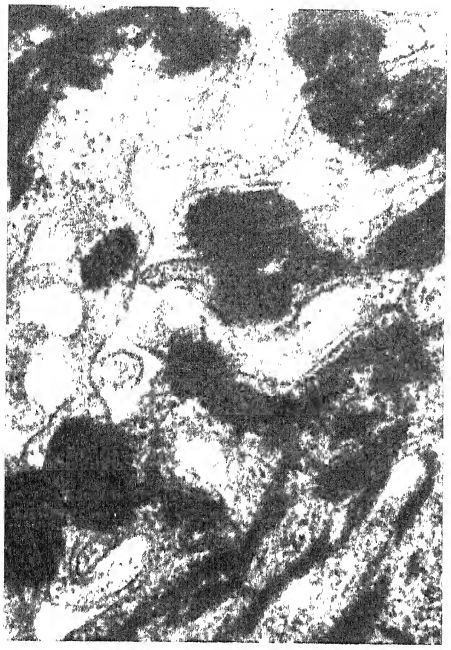
the term is interpreted more widely. It includes the expression and synthesis of keratin proteins in the basal layer cells, their organization into filaments, changes in their chemical composition in the upper layers, and their interaction with keratohyalin granules to form the filamentous-matrix structure of the interior of the corneocyte and strengthening of its envelope. Only a brief summary of these complicated events can be given here.

Keratins are proteins (M.W. 40 000–60 000 Da) of two types, Type I (acidic) and Type II (basic), co-expressed in pairs by epithelial cells (p. 39). Up to 30 different polypeptide chains have been recognized and numbered according to molecular weight. Chains K5 (Type II) and K14 (Type I) are expressed by basal keratinocytes and first of all assembled into two-chain coiled-coil protofilaments, then organized progressively into 4, 8 and 32 chain complexes to form the 10 nm intermediate filaments of the cytoskeleton of the basal cell. Filaments attach peripherally to the desmosomal plaque, and centrally may enter the nuclear pores. New keratin pairs, 1 (Type



5.14B. Electron micrograph of a keratinocyte from the stratum spinosum showing details of its cytoplasmic and nuclear structure and the desmosomal attachments between cells. Magnification × 14 000.

5.15 Stratum granulosum and stratum corneum. The uppermost cell of the stratum granulosum is overlain by the flattened squames of the stratum corneum. Note dense irregular keratohyalin granules in the cytoplasm of the granulosa cell. The keratinized cells of the stratum corneum are devoid of nucleus or organelles, but their internal structure is clearly not identical in all layers. Here in the five deepest layers shown, three variations of internal pattern are seen. Magnification × 20 500.

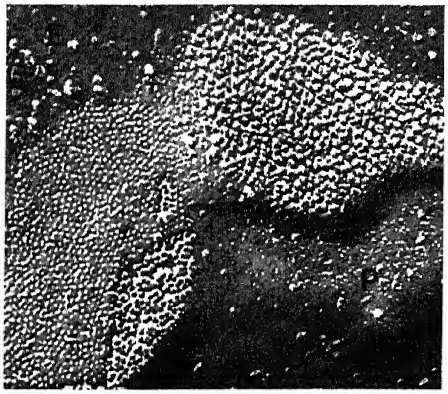


5.16 Desmosomes and cytokeratin bundles from the stratum spinosum. Magnification × 52 500.

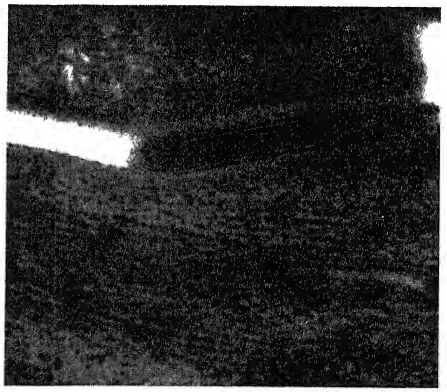
5.17 Keratin filaments attaching to the cytoplasmic aspects of desmosomes. From a keratinocyte of the outer root sheath of a hair follicle. Magnification × 47 250.

11) and 10 (Type I), are synthesized suprabasally, and in the stratum granulosum the filaments are processed into macrofibrils and become associated with keratohyalin granules countaining profilaggrin, a histidine-rich phosphorylated protein. As the cells pass into the stratum corneum, profilaggrin is cleaved by phosphatase enzymes into filaggrin which causes aggregation of the filaments and forms the matrix in which they are embedded. As already mentioned, various patterns of filament-matrix organization within thin sections of corneocytes have been illustrated by different authors, and even in the same section different patterns may be seen in individual cells at different levels. These variations may be due to differences in processing, or to the fact that cells do not enter the stratum corneum synchronously or at exactly the same degree of terminal differentiation. This makes it difficult to define exactly what is the final 'keratin pattern'. In freeze-fracture preparations (5.22) a picture of filaments more or less uniformly distributed within the matrix is seen.

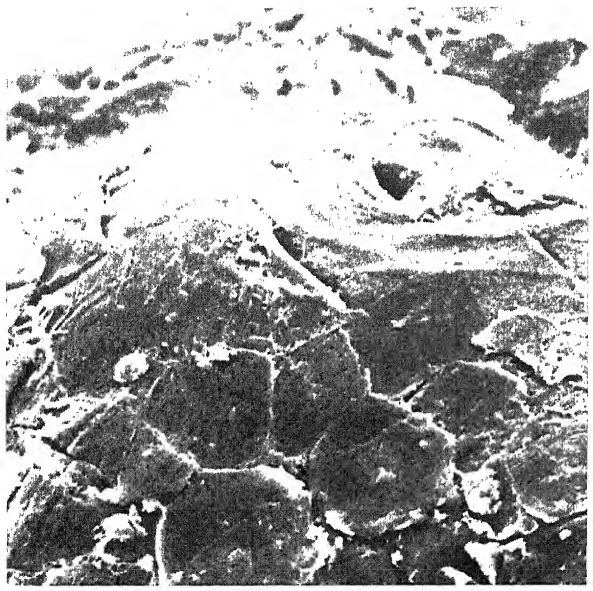
The envelope of the corneocyte consists of a modified plasma membrane strengthened on its deeper aspect by a marginal band. Freeze-fracture (Breathnach et al 1973) reveals that intramembranous particles (IMPs) present on fracture faces of plasma membranes of cells of lower strata are generally absent from fracture faces of the corneocyte membrane, and the manner of fracture of the intramembranous component of the desmosome is also different. IMPs represent transmembrane proteins of the plasma membrane, often associated with the passage of ions and small molecules, and their absence within the corneocyte membrane could indicate that it is metabolically inert. The dense marginal band internal to the plasma



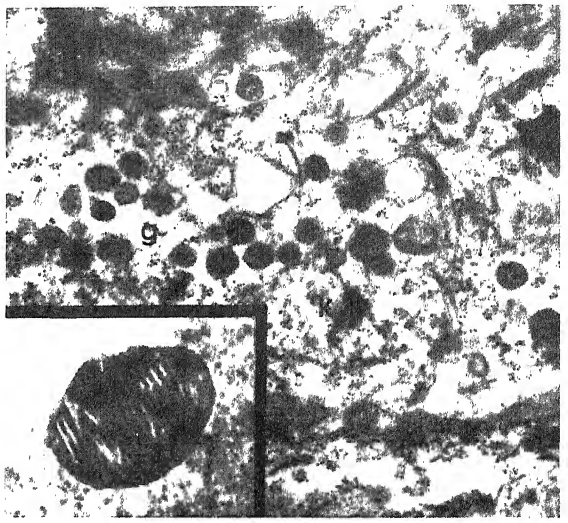
5.18A. Freeze-fracture replica of cellular contact in stratum spinosum. E and P are complementary fracture faces of the plasma membranes of the apposed cells with, on the right of the E face, aggregated particles at the site of a desmosome. Associated with it is a gap junction, defined by closely-packed particles on the P face of one cell membrane, and complementary pits on the E face of the membrane of the apposed cell. Gap junctions are not often seen in adult epidermis, and when present are nearly always closely associated with a desmosome as here. Magnification × 12 500.



5.18B. Stratum corneum. A modified desmosome is seen between two cells with an apparent empty intercellular space on either side. However, other techniques reveal that the intervals between the corneocytes are occupied by organized lipid material derived from lamellar granules. The arrangement of internal filaments shown here is close to that commonly accepted as the typical 'keratin pattern' of the corneocyte, but in fact, it is the one least frequently seen by most observers. Magnification × 32 000.



5.19 Scanning electron micrograph of a portion of the epidermal surface surrounding the aperture of a sweat duct. Several scale-like corneocytes, polygonal in form, are visible. Magnification ×2000.




5.20 Lamellar granules (g) in cytoplasm of cell of stratum granulosum. Keratohyalin granule (k). Magnification ×23 625. Inset: granule stained by the osmium-iodide technique to show internal lamination. Magnification ×165 000. (Reproduced from Breathnach et al 1973, with permission.)

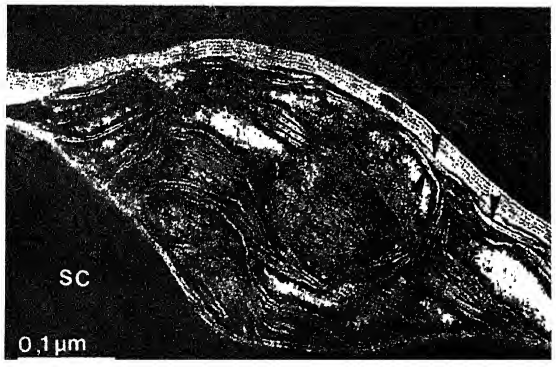
membrane results from cross-linking of a soluble precursor protein, *involucrin*, with membrane associated proteins, catalysed by a transglutaminase. It and the plasma membrane together measure 20 nm in thickness.

Other types of terminal keratinization occur elsewhere, particularly in hair and nails, where the keratin is chemically distinct and becomes much tougher than in the general epidermis ('hard keratin' as opposed to 'soft keratin'). These processes will be considered further, below (pp. 402, 408).

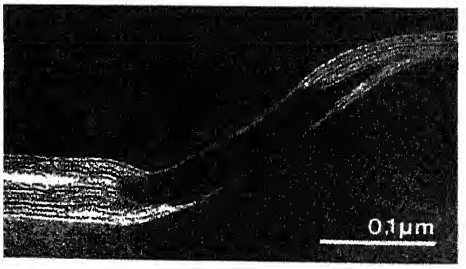
## Extracellular compartment, permeability, barrier function and epidermal lipids

The epidermis serves as an important barrier to the loss of water and other substances through the body surface (apart from sweating and sebaceous secretion), and to their permeation from without. Theoretically, two routes of passage are available, transcellular and extracellular. In the viable strata of the epidermis the plasma membrane provides a generally effective barrier against transcellular transfer, although gap junctions provide ionic and electrical coupling between cells, important in their interactions, especially during development.

Below the stratum granulosum, the extracellular compartment appears on electron micrographs as a lucent 20 nm interval interrupted only by the intermediate components of desmosomes and the occasional gap junction. It is not, however, 'empty', but is composed of the outer glycocalices of the apposed cells containing cell surface receptors, and other molecules, and extracellular proteoglycans and ligands concerned with a variety of regulatory activities and cell cell adhesion. Water soluble tracers, such as horseradish peroxidase (HRP), injected into the dermis can freely traverse the extracellular compartment as far as the upper levels of the stratum granulosum, but not beyond. This is the level at which lamellar granules extrude their contents and at which incomplete tight junctions are present, and it represents the deep limit of the water barrier. Experiments measuring diffusion of water from the exterior show that the entire stratum corneum provides an effective though not complete barrier. Some water soluble substances can traverse it along a polar route, probably directly through the corneocytes, and hydration makes the stratum much more permeable. Lipid-soluble substances can penetrate it more effectively, and this indicates that the water barrier must be primarily lipid in nature. It is, in fact, composed of intercellular glycolipid sheets or lamellae derived from the lamellar granules of the stratum granulosum (Breathnach et al 1973; Elias & Friend 1975; Elias 1983; Landmann 1988; Fartasch et al 1993) which are clearly seen in freeze-fracture preparations (5.22), and in thin sections of ruthenium red-fixed tissues (5.21). The contents of lamellar granules are liberated into the extracellular compartment in the form




5.21A. Uncoiling prelaminar lipid sheets (arrowheads) derived from lamellar granules in the intercellular space of the stratum corneum (sc). Magnification ×73 250.




5.21B. Lipid lamellae on either side of a desmosome in the intercellular space of the stratum corneum. Magnification ×172 500. $RuO_4$ staining. (Reproduced from Fartasch et al 1993, with permission.)


5.22 Freeze-fracture replica of stratum corneum showing lamellated material in intercellular space between fracture faces E and P of two corneocytes. In the stratum corneum, general fracture faces of the cell membranes do not exhibit intramembranous particles except at desmosomal sites (D). Fractured cytoplasm (C) of lower cell shows only filaments. Magnification × 121 500. (Reproduced from Breathnach et al 1973, with permission.)

of discs each containing two apposed lipid bilayers. After extrusion, the discs fuse edge to edge, and are chemically remodelled to form broad, multilamellar sheets throughout the extracellular compartment of the stratum corneum (**5.20–22**). The cornecytes, together with their own lipid envelopes, can, therefore, be regarded in a sense as bricks individually embedded in a lipid cement.

**Epidermal lipids.** A variety of lipids are present and synthesized in the epidermis, including triglycerides and fatty acids, phospholipids, cholesterol, cholesterol esters, glycosphingolipids and ceramides. Apart from their role in barrier formation, lipids, and especially phospholipids, are important components of plasma membranes and membrane-bound organelles, and serve in transmembranal signalling processes. An intermediate in the synthesis of cholesterol, 7-dehydrocholesterol, is the precursor of vitamin D. The content and composition of epidermal lipids changes with differentiation. Phospholipids and glycolipids at first accumulate within keratinocytes above the basal layer, but higher up they are broken down and are practically absent from the stratum corneum. Cholesterol, its esters, fatty acids and ceramides also accumulate towards the surface, and are abundantly present in the stratum corneum. Whereas the lamellar arrangements of the extracellular lipids is a major factor in their barrier function, it is not clear how important the exact species composition of the sheets is. Clearly, it will be important to establish the role of individual lipids in this respect, especially in connection with delivering drugs across the barrier. Stratum corneum lipids may participate in a variety of other functions such as antimicrobial activity, antioxidant generation and xenobiote metabolism, and recognition of this is leading to a reappraisal of the stratum corneum as not just a passive, inert, selectively permeable layer, but one in which some catabolic and enzymatic activities may continue (Elias 1989). For reviews of topics referred to in this section, see Bronaugh and Maibach (1989), Maibach and Downing (1992), and Rawlings et al (1994).

## Epidermal keratinocyte population dynamics

The epidermis is a cellular system undergoing continuous renewal, where, in order to maintain a constant normal thickness, the rate of cell production must equal their loss. Disturbances in division and maturation rates are common in various skin diseases, so a knowledge of the mechanisms of normal cell population dynamics (*kinetics*) is important (Davis & Wright 1991). The subject is also of importance in development wound healing and ageing of skin (see pp. 411, 412–418).

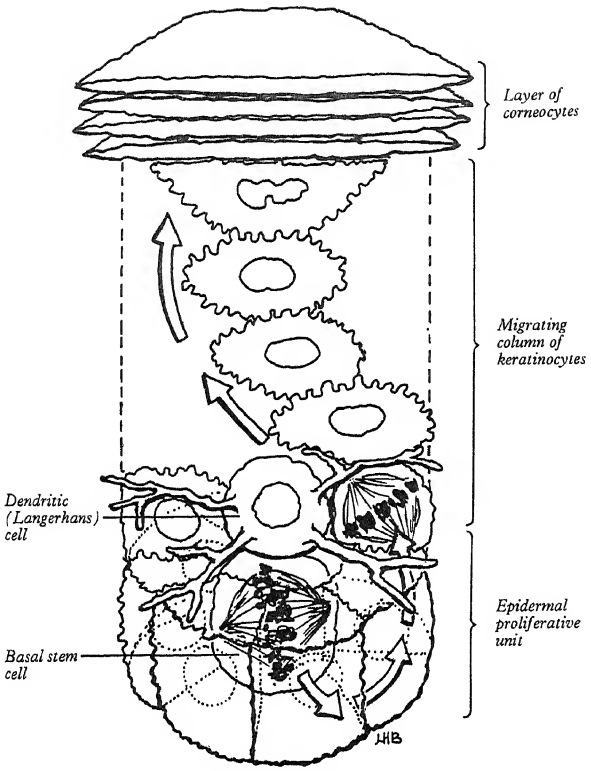
At any one time in the basal layer of the epidermis there is a variety of keratinocytes in different states of differentiation. There are *stem cells* (10%), which on division produce one of their like to maintain production, and another daughter cell, which can have a variable fate:

- it can remain for a while in the basal layer undergoing a limited number of mitoses, when it is known as a *proliferative cell* (60%) undergoing *amplification* (*transit*) divisions;
- it may remain quiescent in the basal layer for a time as a $G_o$ cell which will resume mitosis later (see p. 23);
- it may lose the ability to divide, remaining for a time in the basal layer as a *postmitotic maturing cell* (40%).

Stem cells and proliferative cells are *recycling cells*, and postmitotic maturing cells are *non-cycling cells*; $G_0$ cells occupy a somewhat equivocal position in this classification, since for a time they are non-cycling, but ultimately re-enter the cycle of production as proliferative cells. As a result of this activity of stem cells and proliferative cells in the basal layer, a continuous supply of non-cycling postmitotic maturing cells is passed on to the spinous layer, traversing this as *differentiating cells*, then moving into the granular layer and stratum corneum, to be finally desquamated at the surface. These layers are known as *transit compartments*, though some cell division does occur in the more basal regions of the stratum spinosum. Stem cells are thought to reside mainly at the tips of the rete pegs, and in the outer root sheath and bulge of the hair follicle, but they cannot easily be distinguished morphologically from their neighbours.

Cell proliferation kinetics can be given a degree of quantitative expression by measuring certain parameters. Techniques which have been employed include administration of colchicine to arrest mitosis, pulse-labelling with [$^3$H]-thymidine and microradioautography, and immunocytochemical detection of 5-bromodeoxy-uridine (BUdR) incorporation. With these methods, the following can be measured: *cell cycle time*, the interval between a stem cell mitosis and the next mitosis of its daughter cell(s); *proliferative index* (mitotic index or flash-labelling index) which measures the proportion of basal cells in cycle; *turnover time*, which is the time taken to replace all cells in a compartment; *transit time*, the time it takes a cell to traverse a compartment; and *total epidermal turnover time*, the time taken for a non-cycling cell to pass from the basal layer to desquamation from the surface. Widely varying estimates of these parameters derive mainly from measurements on animals, and reliable figures for human epidermis are few. The normal total epidermal turnover time is quoted as between 52 and 75 days. Rates and times for the various parameters can vary with region, epidermal thickness, degree of skin abrasion, ambient temperature, and time of day. Circadian rhythms in the S-phase and M-phase of the mitotic cycle (p. 22) have been demonstrated. In rodents, the S-phase peaks at 3.30 a.m., and the M-phase at 8.30 a.m.; in humans, the S-phase peaks at 3.30 p.m., and the M-phase at 11.30 p.m. (Brown 1991). In some pathologies of skin, turnover rates and transit times can be exceedingly rapid; for example, in psoriasis, total epidermal turnover time may be as little as 8 days, and in such conditions differentiation of supra-basal layers is disordered, the stratum corneum does not keratinize properly and the barrier functions of the skin break down.

Potten's (1975, 1983) proposal of an *Epidermal Proliferative Unit* (EPU) seeks to unite state and rate parameters in a single concept of kinetic organization which can be demonstrated histologically. If the epidermis of some mammalian species (e.g. rats) is treated with dilute alkali before sectioning, the cells of the stratum corneum swell, and can then be seen to be stacked in regular columns (**5.23**). Beneath each column are stacked several layers of spinous and granular cells overlying a group of six to eight basal cells, each group consisting of a central stem cell with an encircling ring of what are essentially either proliferative or postmitotic maturing cells (see above). From the periphery of this ring, non-cycling cells are fed into the stratum




5.23 The concept of the epidermal proliferative unit and its relationship to the overlying column of differentiating keratinocytes, finally maturing through the stratum granulosum into the flattened corneocytes of the stratum corneum. In this model, keratinocytes arise by the repeated mitosis of a single basal stem cell, move laterally, then pass into the base of the stratum spinosum, where they may divide again one or more times before passing towards the surface. A single basal stem cell and its progeny within the stratum basale constitute the epidermal proliferative unit as initially conceived. The dendritic Langerhans cell, situated above the basal stem cell, has also been suggested as a controlling influence on cell division. For further explanation see text.

spinosum. The whole forms a cellular prism, the EPU, and each unit is a discrete entity, i.e. not feeding cells laterally into adjacent units. Whereas this arrangement seems to apply to species and sites where epidermal cell turnover is regular and not too fast, regular columns are rarely demonstrable in human epidermis, and it is questionable to what extent the concept applies here. Potten originally proposed that each unit contained a single Langerhans cell which directed mitotic activity within it, but though it can affect keratinocyte growth it is doubtful if it plays the predominant role suggested.

Under normal conditions the production of epidermal keratinocytes is matched by loss of cells from the stratum corneum, and this dynamic equilibrium is achieved by a balance of stimulating and inhibiting signals regulating proliferation of basal layer cells. Among growth- and diffusable factors which stimulate cell proliferation and differentiation are Epidermal Growth Factor (EGF), GkDa polypeptide, Transforming Growth Factor (TGF)-$\alpha$, cytokines, and Basic Fibroblast Growth Factor (b-BFGF). Some of these are secreted by the basal keratinocytes themselves. Growth inhibitors include TGF-$\beta$, pentapeptide, and $\alpha$- and $\gamma$-interferons. *Chalones* are substances thought to be secreted by suprabasal cells which bind to adrenaline and inhibit basal layer mitosis by a negative feedback mechanism, though evidence for their existence is mainly circumstantial. Recently, physiological cell death (*apoptosis*) has been recognized as an active regulatory mechanism in shaping and maintaining tissue size, and may play a role in normal epidermal kinetic homeostasis.

Hormones—androgens, oestrogens, corticosteroids—and vitamin A and its metabolites, the retinoids, can also affect keratinocyte turnover and differentiation, and Langerhans cells and lymphocytes are increasingly being thought of as accessory regulators in the context of a triad of cells forming a peripheral monitoring immunological unit. Extracellular matrix components of the dermis also influence epidermal cell population dynamics, especially during development, through interactions at the basement membrane zone (BMZ) (see p. 397), and reduction of differentiating keratinocyte adhesiveness to extracellular matrix proteins, including fibronectin, facilitates their migration out of the basal layer (Nicholson & Watt 1991). Following binding of the various mediating factors to keratinocyte surface receptors, intracellular signals are triggered by 'second messengers' (see p. 24); also Elder et al 1991). Changes in keratinocyte cell to cell adhesivity during suprabasal differentiation and stratification also involves changes in the expression of adhesion molecules, such as cadherins, integrins, etc.

Clearly, separation and migration of cells to other layers involves disruption, reformation and relocation of desmosomes along the plasma membrane. Freeze-fracture replication reveals the presence of aggregated intramembranous particles composed of glycoproteins (desmogleins) within the plasma membrane at desmosomal sites (Breathnach et al 1972; Caputo & Perluchetti 1977). In fetal epidermis (Breathnach 1973), extensive areas of desmosmal particles are seen from which individual aggregates seem to bud. This suggests that certain sites on the plasma membrane may be coded for desmosome formation by lateral movement of specialized particles within the plane of the membrane, and a similar mechanism might account for their relocation and redisposition associated with asynchronous movements of previously apposed cells. Calcium could be involved as mediator in this process: addition of calcium to cultures of keratinocytes causes redistribution of desmosome-associated proteins and induces desmosome formation (Hennings & Holbrook 1983), and transmembrane desmosomal glycoproteins can bind to calcium. Calcium may also be otherwise involved in cell to cell adhesion mechanisms since it binds to the extracellular regions of the group of cell adhesion molecules known as cadherins (Menon et al 1994).

Melanocytes are not involved in the movement of keratinocytes to upper levels, maintaining station along the basal lamina. This could be due partly to lack of desmosomes linking them to adjacent keratinocytes, and to the maintenance of adhesiveness to basal lamina fibronectin via integrin binding. Langerhans cells also manage to maintain position within the lower epidermal levels despite the general superficially directed current of the stream of movement, and indeed they can move against it in proceeding downward into the dermis. They might be regarded as floating or swimming in the stream, and their dendritic shape might result from, or perhaps help in, this movement.

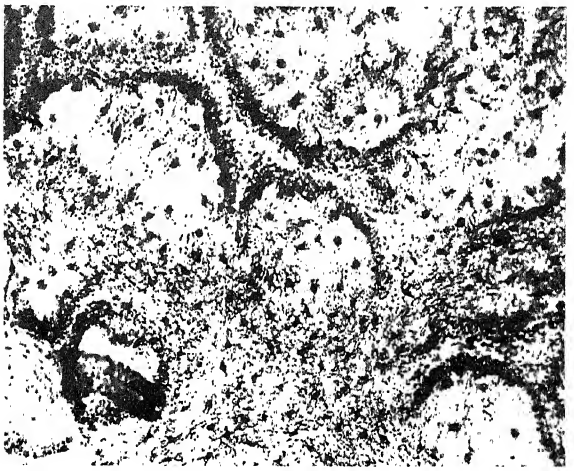
## EPIDERMAL MELANOCYTES AND SKIN PIGMENTATION (5.24–30)

Melanocytes are *melanin-pigment* forming cells derived from the neural crest and widely distributed throughout the body in vertebrates. In humans, they are present in the epidermis and its appendages, in oral epithelium, some mucous membranes, the uveal tract (choroid coat) of the eyeball, parts of the middle and internal ear and in the leptomeninges at the base of the brain. The cells of the retinal pigment epithelium, developed from the outer wall of the optic cup, also produce melanin, and neurons in different locations within the brainstem (e.g. the locus coeruleus and substantia nigra) synthesize a variety of melanin called neuromelanin.

True melanins are complicated high molecular weight polymers attached to a structural protein (to form melanoproteins), and in humans there are two classes, the brown-black *eumelanin*, and the red-yellow *phaeomelanin*, both derived from the substrate *tyrosine* by a complicated series of reactions initially catalysed by the enzyme *tyrosinase* (see p. 391). *Melanophages* are macrophages which have ingested preformed melanin, and *melanophores* are dermal melanocytes, especially common in fishes, amphibians and reptiles, though absent in humans, within which melanin can be rapidly aggregated or dispersed to change body colour in adaptation to environmental backgrounds.

Embryonic precursors of melanocytes (melanoblasts) migrate from the neural crest to enter the epidermis as melanocytes from about the eighth gestational week (Sagebiel & Odland 1972), and by the 14th week may have reached densities of 2000/mm$^2$ in some regions,

5.24 Section of skin incubated in 1 in 1000 buffered DOPA solution. Dendritic DOPA-positive melanocytes are present in the basal layer of the epidermis and stain black (arrowheads) because they contain the enzyme tyrosinase. Other cells of epidermis and dermis are DOPA-negative. The fact that faint outlines of epidermis and dermis are seen is due to non-specific staining of the solution due to auto-oxidation of DOPA during the reaction. Magnification ×320. (Reproduced from Breathnach 1960, with permission.)



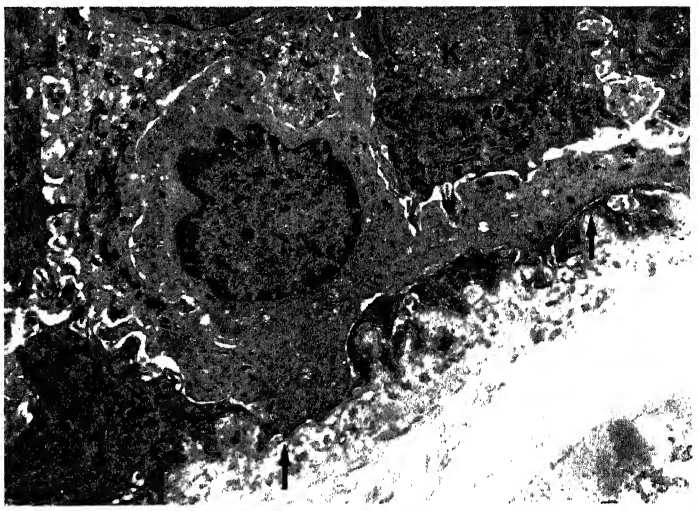
5.25 'Split-skin' sheet of epidermis which was incubated in DOPA solution, viewed from the deep surface. DOPA-positive dendritic melanocytes are seen, and it is from this type of preparation that estimates of their population density per unit area of epidermis can be obtained. Not all cells are in focus because of the undulant nature of the epidermal–dermal junction, and the rete ridges of the epidermis are outlined by melanocytes superimposed at different levels along them, as well as by some melanin pigment. Magnification ×145.

partly by division of cells in situ. Shortly after their arrival, the cells engage in melanogenic activity, indicating that melanin has functions (as yet poorly understood) other than protection against solar radiation. In some oriental races, melanocytes persist for a while postnatally in the dermis of the lumbar region and buttock giving rise to bluish *Mongolian spots*. The blue colour is due to scattering of blue light by collagen bundles overlying the melanin-containing cells. In routine histological preparations stained with haematoxylin and eosin, melanocytes appear as 'clear cells' in the basal layer of the epidermis; they can be stained with Masson's ammonium silver nitrate method, but are best revealed in sections and in 'split-skin' pure epidermal sheets, by the DOPA technique (5.24, 25). This involves incubation in a buffered 1 in 1000 solution of DOPA (dihydroxy-phenylalanine) when the dendritic nature of the cells is well visualized. They can also be recognized in fetal epidermis by the monoclonal antibody HB-45 (Holbrook 1989). Cell counts on DOPA preparations reveal considerable regional variations in numbers of melanocytes per unit area of epidermis, ranging from 2300 per $mm^2$ in the cheek to 800 per $mm^2$ on the abdomen (Szabo 1959). It is estimated that a single melanocyte may be in functional contact via its dendrites with up to 30 keratinocytes to form an entity called the Epidermal Melanin Unit (Fitzpatrick & Breathnach 1963). In general, there are no sexual or racial differences in frequency distribution of melanocytes, and intrinsic or acquired variations in melanic pigmentation are due rather to differences in the melanizing activity of the cells than in their numbers. Melanocytes constitute a reproductive self-maintaining system of cells, although their normal turnover rate is very slow and an accurate mitotic balance sheet is lacking. They can be readily cultured in vitro (5.28). When locally depleted, they repopulate the epidermis, and a keratinocyte-derived growth factor, FGF-$\beta$ (Halaban et al 1988), is probably involved as a mitogen. UV radiation also stimulates mitotic activity of melanocytes.

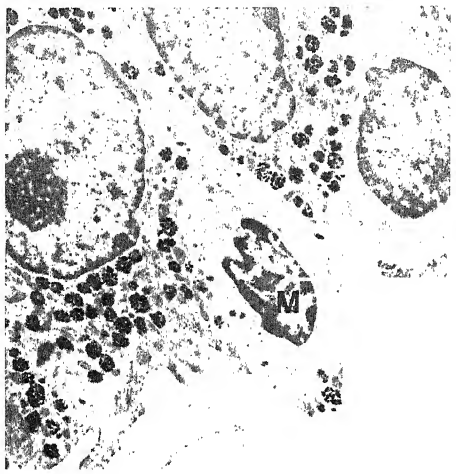
It should be emphasized in evaluating numerical estimates, that the DOPA reaction reveals only synthetically active melanocytes, depending as it does on the presence of the enzyme tyrosinase within the cell. In albinism, where the enzyme is either absent or blocked, melanocytes, though present, are not stained with DOPA, and relatively inactive cells in the normal epidermis may be missed through giving a weak reaction. Melanocytes decrease significantly in numbers in old age, and are absent from grey-white hair.

## Ultrastructure of melanogenesis

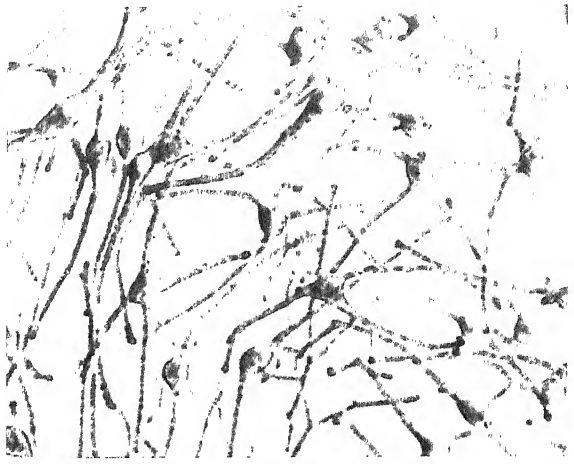
Like the Langerhans cell, the melanocyte is a dendritic non-keratinocyte (5.25), lacking desmosomal contacts with apposed keratinocytes, though hemidesmosomal contacts with the basal lamina are present. The nucleus is large, round, and euchromatic, and in the cytoplasm, are: intermediate filaments, a prominent Golgi complex and vesicles and associated granular endoplasmic reticulum, mitochondria, and coated vesicles, together with a characteristic marker organelle, the *melanosome* (5.26, 27). The melanosome is a membrane-bound structure which undergoes a sequence of four developmental stages during which melanin is synthesized and deposited within it by the tyrosine–tyrosinase reaction. The *Stage I melanosome* is a spherical vacuole, derived probably from the rough endoplasmic reticulum, and containing filamento-amorphous structural protein and vesiculoglobular bodies. Subsequent stages of eumelanosomes and phaeomelanosomes differ somewhat in morphology (5.29A, B). *Stage II eumelanosomes* become spherical or




5.26 Basal epidermal melanocyte showing general ultrastructural features. There are no desmosomes connecting it with apposed keratinocytes (K), the cytoplasm contains no keratin fibrils, and melanosomes are present as individual granules in the cytoplasm. Note dendrite extending towards the right. Arrows mark the epidermal–dermal junction. Magnification ×5625.



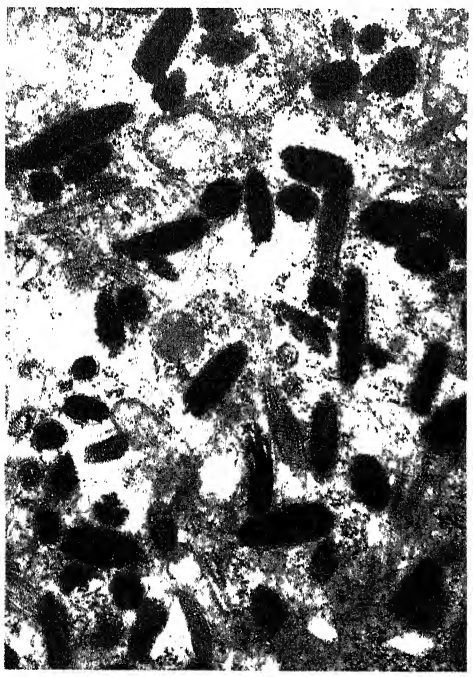

5.27 Melanosomes aggregated in groups in basal keratinocytes. Note few melanosomes in melanocyte (M). It produces the melanosomes and passes them on to the keratinocytes. Magnification × 4950.



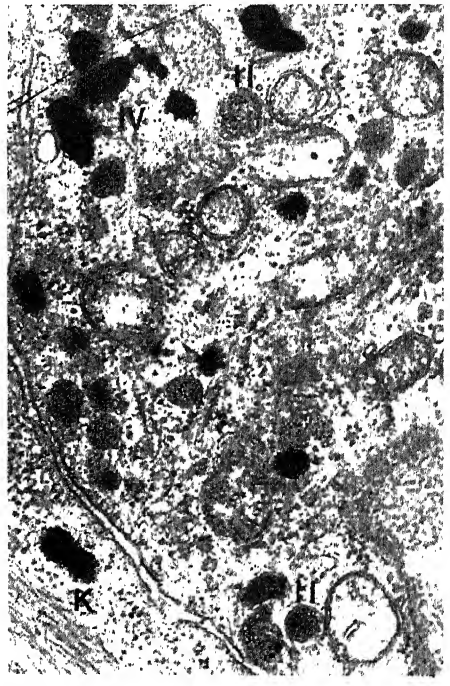
5.28 Pure culture of melanocytes from infant foreskin. In culture the cells retain their dendritic shape. Magnification × 450.

ellipsoid and the inner matrix becomes organized into filamentous sheets exhibiting a 9 nm periodicity. At *Stage III*, melanin begins to be deposited on the inner sheets, gradually obscuring their arrangement, until the final, densely-pigmented *Stage IV* is reached, exhibiting no other internal structures apart from non-melanized vesiculoglobular bodies. *Phaeomelanosomes* retain their spherical shape throughout all stages, their inner matrix is not organized into sheets, and at Stage IV a microgranular internal structure may still be apparent. In most active melanocytes, melanosomes of all stages are present; in albinos, Stages I and II are present, though the inner matrix may lack its typical organization. When mature, Stage IV melanosomes move into the dendrites along the surfaces of microtubules and are transferred to keratinocytes by a special type of phagocytosis involving the latter cell nipping off and internalizing the tip of the dendrite with the subsequent liberation of melanosomes into the keratinocyte cytoplasm (5.27). Here, they may exist as individual granules in heavily-pigmented skin, or be packaged within secondary lysosomes as melanosome complexes or compound melanosomes in lightly pigmented skin. They are often aggregated above the nucleus to form supranuclear caps. As the keratinocytes progress towards the surface, melanosomes undergo degradation, and melanin remnants in the stratum corneum are more in the form of dust-like particles.

Lipid droplets frequently occur in normal melanocytes, and in the eyelid there is a melanocyte which contains them in abundance, and which is known as the 'unicellular sebaceous gland of Wolff' (Pelfini et al 1969).



5.29A. Cytoplasm of hair bulb melanocyte containing melanosomes at Stages II and III. From the elongated rod-shaped form, these are identified as eumelanosomes. K, keratinocyte. Magnification × 32 550.




5.29B. Cytoplasm of melanocyte containing melanosomes mainly at Stages II and IV. From the predominantly round sectional profiles and granular internal structure, these are identified as phaeomelanosomes. Magnification × 51 000.

**Tyrosinase, and synthesis and properties of melanins.** Tyrosinase is a copper containing metallo-enzyme, present in the form of several isozymes, which catalyses the initial stages of the synthesis of tyrosine-melanin. It is formed by ribosomes on the granular endoplasmic reticulum, conveyed to the Golgi complex, glycosylated and incorporated into coated vesicles which attach to the limiting membrane of the Stage I melanosome, liberating the active enzyme into its interior. Melanization then commences via a complicated pathway the complete details of which remain to be worked out, and which differ between eumelanin and phaeomelanin. The first two steps, oxidation of tyrosine to DOPA, and DOPA to dopaquinone, are common to both, and catalysed by tyrosinase. Next, in eumelanin synthesis, dopachrome is formed, converted into dihydroxyindoles and 5–6 dihydroxyindole-2-dicarboxylic acid by a mechanism involving a conversion factor and possibly two enzymes, dopachrome oxido-reductase, and dopachrome tautomerase, though these latter may be one and the same (Pawelek 1991). The final stages in the pathway to melanin essentially involve complex polymerizations in which tyrosinase may again be involved. In phaeomelanin synthesis, the amino-acid cysteine is added to dopaquinone to form 5-S cysteinyldopa. Evidence is increasing that most natural melanins are mixtures of eumelanin and phaeomelanin, and phaeomelanic pigments, *trichochromes*, occur in red hair.

Melanin has biophysical and biochemical properties related to its functions in skin. It protects against damaging effects of UV radiation on DNA through its spectral absorptive electron–photon coupling, and amorphous semiconductor properties, whereby it can absorb many different types of energy and dissipate them in the form of vibrational modes or heat. Its redox capacity makes it an efficient scavenger of damaging free radicals, however generated, and its ability to bind to a variety of metal ions and drugs suggests it can act as an antitoxic agent. However, if the energy input is too great, these properties can be expressed in the output of toxic activated chemical species which can be damaging (Hill 1992). Another disadvantage is that a high concentration of melanin in relation to incident solar UV may adversely affect synthesis of vitamin D.

## Determination and control of melanin pigmentation

Melanin pigmentation of human skin can be analysed on two bases: *constitutive* and *facultative*. Constitutive pigmentation is the intrinsic level, genetically determined, and facultative pigmentation comprises reversible changes induced by environmental agents, for example UV and X-radiation, chemicals, and hormonal influences.

**Genetics.** In rodents, at least 130 genes at 50 loci are known to determine skin and coat melanization, acting not only on the melanocytes themselves, but also on their environment. Specific genes can influence differentiation of neural crest cells into melanoblasts, and also melanoblast migration to the skin, their differentiation into melanocytes, and morphological features of these latter, such as shape, size and length of dendrites, which in turn determine the size of the pool of keratinocytes to which each cell transfers its melanosomes. Other genes acting primarily within the melanocyte control the synthesis of tyrosinase, its type and activity (including inhibitors), the type of melanin synthesized, the size, shape, protein structure and number of melanosomes, their degree of melanization, and their rate of transfer to keratinocytes. Constitutive melanin pigmentation in man is probably under similar precise genetic control, though positive evidence is not easy to obtain due to co-mingling of genes. Various types of *albinism* are genetically determined.

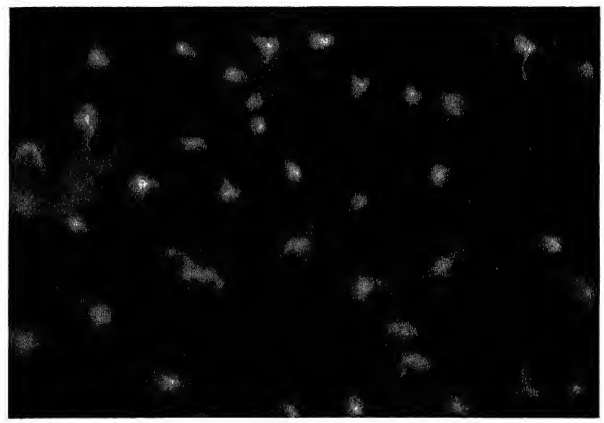
Racial variations in pigmentation are due to differences in melanocyte morphology and activity rather than to numerical differences. In naturally heavily pigmented skins the cells tend to be larger, more dendritic, and to contain more and larger Stage III and IV melanosomes than melanocytes of paler caucasoid skins. The keratinocytes in turn contain more melanosomes, individually dispersed, whereas in caucasoids the majority are contained within secondary lysosomes to form melanosome complexes. (Melanosomes occasionally seen within Langerhans cells are always similarly enclosed.) For more on this topic, see Robins (1992).

**Ultraviolet irradiation.** *Photobiology* is a rapidly expanding discipline concerned with the overall effects of solar irradiation, and in particular, UV irradiation, on the skin (see Fitzpatrick et al 1974; Potten 1985; Posschier et al 1987). The response of the melanin pigmentary system to UV varies with genetic and constitutional factors. It includes *immediate tanning*, or *pigment darkening*, which can occur within a matter of minutes, probably due to photo-oxidation of pre-existing melanin. *Delayed tanning* occurs after about 48 hours, and involves stimulation of new melanogenesis within the melanocytes, and transfer of additional melanosomes to keratinocytes. There may also be some increase in size of active melanocytes, and in their apparent numbers, both through division, and activation of dormant cells. Keratinocytes can be directly involved in these changes through various signals. In vitro, the lower frequency end of the UV band (UVB) induces synthesis by keratinocytes of b-FGF, which is mitogenic for melanocytes, as well as Interleukin I which induces them to produce $\alpha$-melanocyte stimulating hormone ($\alpha$-MSH), a known stimulant of melanogenesis (see below); there is evidence that keratinocytes may also produce adrenocorticotrophic hormone (ACTH). Chronic exposure to UV results in changes dealt with below under 'Age related changes in skin' (p. 411).

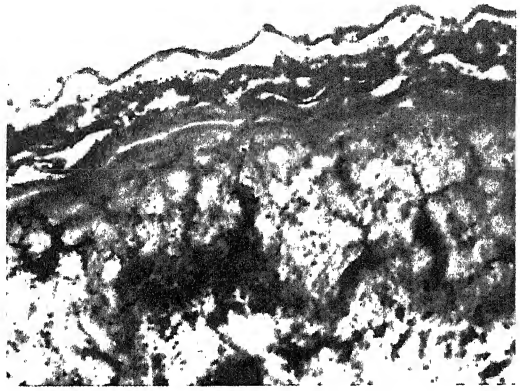
*Freckles* in skin of red-haired individuals are usually thought to be induced by UV, though they do not appear initially until several years after birth, despite exposure. Paradoxically, melanocytes are significantly fewer in freckles than in adjacent paler epidermis, but they are larger and more active. What determines the onset of freckles, or their individual location, is not known.

**Hormonal influences.** In amphibians, MSH from the anterior lobe of the hypophysis, and melatonin, a skin-lightening hormone secreted by the pineal, are involved in pigmentary alterations, though their importance as normal regulatory factors in man is unclear. When administered to humans, $\alpha$-MSH causes an increase in pigmentation due to a cyclic adenosine monophosphate (cAMP)-mediated increase in tyrosinase activity, and as mentioned above, UV irradiation induces MSH production by keratinocytes. ACTH is also thought to affect melanocyte activity, and is probably responsible for the hyperpigmentation associated with pituitary and adrenal disorders. The role of melatonin in the biology of human melanin pigmentation remains problematic. In pregnancy, higher levels of circulating oestrogens and progesterone are responsible for the increased melanization of the face, abdominal and genital skin, and the nipple and areola, and much of this may remain permanently. A number of other factors operating within the epidermis, such as interleukins, arachidonic acid, prostaglandins and various cytokines, also affect melanogenesis, either stimulating or inhibiting individual steps, or inhibiting natural tyrosinase inhibitors within the melanocyte. Clearly, the level of pigmentation at any given time represents a balance between a large number of competing influences, constitutive and facultative, and these must be taken into account in the analysis and diagnosis of hypo- and hyperpigmentary disorders.

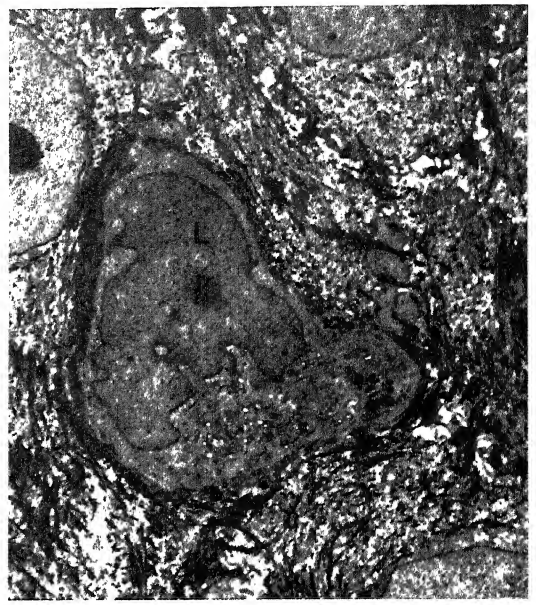
**Summary.** Further details on all aspects of melanin pigmentation will be found in: Quevedo et al (1987), Nordlund et al (1989), Jimbow et al (1991), Prota (1992), Robins (1992), and Takeuchi and Quevedo (1992).



5.30 Epidermal sheet preparation. Immunofluorescence staining with Phycoerythrin anti-HLA-DR showing Langerhans cell network. Magnification x 95. (Provided by Dr S Breathnach, St John's Dermatology Centre, UMDS, St Thomas' Campus, London.)



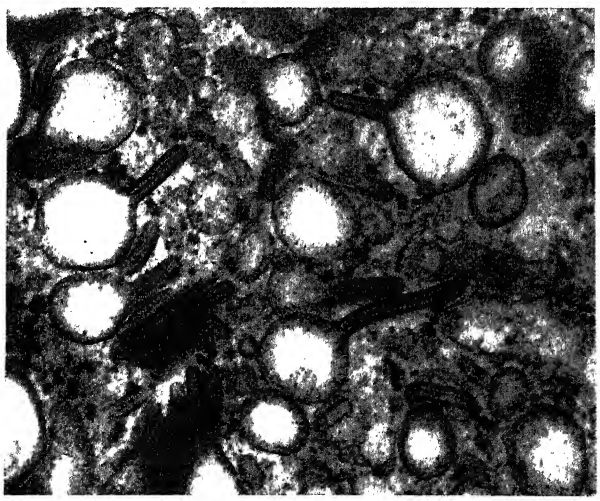
5.31 Section of skin stained with Gairn's gold chloride method. Gold-positive dendritic Langerhans cells are seen at suprabasal levels. Magnification ×315.



5.32 A Langerhans cell (L) in the stratum spinosum of the epidermis. Note indented nucleus, cytoplasm with no keratin filaments which are characteristic of surrounding keratinocytes, and absence of desmosomes along the plasma membrane. The characteristic granules of the cytoplasm (5.33) are too small to be clearly identified at this enlargement. Magnification ×5325.

## Langerhans cell: immunological surveillance

Langerhans cells are dendritic antigen-presenting cells regularly distributed in mammals throughout the basal and spinous layers of the epidermis (p. 381) and its appendages, apart from the sweat gland, and of other stratified squamous epithelia, including the buccal, tonsillar and oesophageal epithelia, as well as the cervical and vaginal mucosae and the transitional epithelium of the bladder. They are present in the conjunctiva, but not in the cornea. In routine H&E preparations they appear as high-level 'clear cells', and with other techniques such as staining with gold chloride or osmium-zinc iodide, their characteristic dendritic shape becomes apparent (5.31). They are also visualized in adenosine 5'-triphosphate (ATP)ase preparations and by a variety of immunofluorescence and immunocytochemical techniques (5.30). They are immunocompetent cells derived from bone marrow (Katz et al 1979), and enter the epidermis as early as the fifth to sixth week. The postnatal population (460–1000/mm$^2$, 2–3% of all epidermal cells, with regional variations) is maintained both by continual renewal from the marrow and, minimally, by division in situ. Following almost complete depletion in rodents by tape-stripping of the epidermis, or X- and UV radiation, normal numbers of Langerhans cells return by 4–15 days. Extra-epithelial Langerhans cells are present in the dermal stroma, occasionally within dermal capillaries and lymphatics, and in lymph nodes, thymus, and spleen (with, of course, a pool of precursors in the bone marrow).



5.33 Langerhans granules. Note rod-shaped sectional profiles with central linear periodicity, and terminal blowing-out of the limiting membrane. At top right, a granule sectioned tangentially exhibits a lattice arrangement of particles. Magnification ×62 010.

**Ultrastructure.** Langerhans cells are non-keratinocytes in that desmosomes are absent from the plasma membrane, the cytoplasm lacks keratin filaments, they do not become keratinized and are not desquamated. The nucleus is euchromatic and markedly indented (5.32), and the cytoplasm contains a well-developed Golgi complex, lysosomes often containing ingested melanosomes, and a characteristic marker organelle, the *Birbeck granule* (Birbeck et al 1961). These structures are discoid or cup-shaped, and on section (5.33) present a variety of appearances. The commonest is that of a rod 0.5 μm long and 30 nm wide, with a linear fuzzy coat within the trilaminar limiting membrane and a central core of two linear arrays of particles with a periodicity of 9 nm. Vesiculation of the limiting membrane at either end gives an appearance similar to a tennis racket and, when sectioned obliquely, an orthogonal or lattice array of the central particles is evident. What appear to be granules may occasionally be seen continuous with the plasma membrane, and this has raised questions as to their origin, nature and function. The bulk of current evidence (Schuler et al 1991; Bartosik 1992; Bucana et al 1992) suggests that the structures attached to the plasma membrane are formed by internalization and zipping of segments of plasma membrane associated with coated vesicles, the resulting structures then undergoing unzipping, vesiculation and fusion with endosomes, to deliver molecules to the interior. There is some doubt as to the exact correspondence of classical, closed, intracytoplasmic granules with those attached to the plasma membrane, which have been referred to as 'Birbeck granule-like structures'. If they are not identical, this allows revival of the original suggestion that Birbeck granules are derived from the Golgi apparatus (see above). Hanau et al (1991) have reported the presence within ethylene diamine tetracetic acid (EDTA)-treated human blood platelets of elements ultrastructurally similar to Birbeck granules and apparently formed by collapse of the *surface-connected canalicular system*. This has led them to suggest that the granules may derive from transformation of vacuolar or canalicular structures due to ligand–receptor interactions while resident in the epidermis. Apart from this, Langerhans cells can exhibit general phagocytic activity, but not to the same extent as keratinocytes. Cells with all the features of Langerhans cells apart from the granules may be present in the basal layer, and are referred

to as 'indeterminate cells'; they are thought to be either immature stages, or else the most mature, about to leave the epidermis (see below). Cells with more electron-dense cytoplasm exhibiting degenerative features and present at all levels of normal epidermis except the striatum corneum (Breathnach 1981) may have been damaged by solar radiation or some other environmental insult, or, perhaps, represent a distinct phenotype or functional stage.

Another dendritic non-keratinocyte, also of bone-marrow origin, the Thy-1 + EC, is present in murine epidermis, and belongs to the T-cell lineage (see Schuler et al 1991). Any analogue of this cell type has not yet been certainly demonstrated in human epidermis.

**Immunoreactivity.** Langerhans cells belong to the general group of dendritic cells (DC), mononuclear phagocytic cells important in immunological reactions (see p. 1415), though their exact relationship to other members of this group, such as 'veiled cells', interdigitating reticulum cells, DC in peripheral blood and lymph, and B-cell related dendritic cells of lymphoid follicles, remains uncertain (Romani et al 1991a,b; Bergstresser et al 1992). Phenotypically, they carry receptors for the Fc portion of IgG, and for complement components (C3b–C4b and C4d), and express a variety of antigens, the number of which is being added to regularly. These include MHC Class I and Class II antigens (Ia; HLA-DR) and CDIa antigen, and the cytoplasm expresses S-100 protein. Preparation of monoclonal antibodies to these and other markers has allowed specification and visualization of Langerhans cells by immunofluorescence and immunocytochemical techniques. The phenotype of human Langerhans cells differs in some respects from that of experimental animals, and in cell culture is significantly altered, becoming more like that of dendritic cells within lymphoid tissue. The capacity of the cell to produce Birbeck granules is reduced in culture, and this, together with the change in phenotype, is regarded as a badge of 'maturity' in terms of ability to sensitize T-cells (see below). Implicit in this concept is the suggestion that the majority of resident Langerhans cells are immature from a functional point of view.

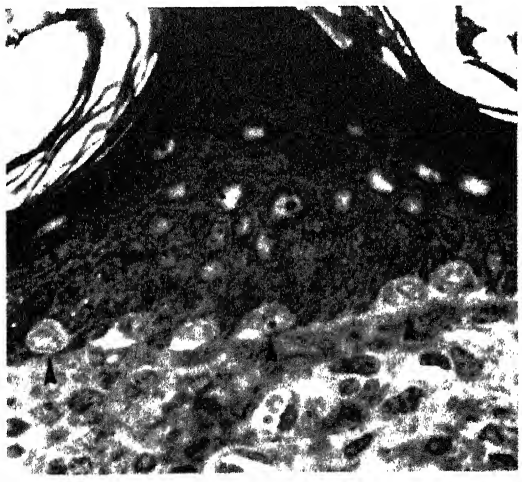
**Functions.** The Langerhans cell is now recognized as a key element in a Skin Associated Lymphoid Tissue (SALT) (Streilein 1983), which is the peripheral outpost of the body's immune surveillance system and involved in induction and regulation of the primary immune response. SALT comprises the Langerhans cell, T-lymphocytes and keratinocytes, together with local draining peripheral lymphatics and associated lymph nodes. In this scheme, the Langerhans cell, under appropriate conditions of normal monitoring, or in the course of a contact hypersensitivity reaction, internalizes antigen (haptens, proteins, local tumour antigens, etc.) by receptor-mediated endocytosis via 'Birbeck granule-like structures' (Bucana et al 1992), processes it, becoming 'mature' meanwhile, then migrates to the draining lymph nodes to present it to unstimulated T-cells. It initiates antigen-specific T-cell activation and proliferation, and generation of cytotoxic T-cells (see p. 1420). These activities involve a traffic in both directions of Langerhans cells across the epidermal–dermal junction, their presence, especially in contact hypersensitivity reactions, in dermal lymphatics and nodes, and their apposition to lymphocytes within the epidermis. A positive regulatory role for the keratinocyte in SALT is becoming increasingly apparent. It also expresses Ia antigen, produces cytokines which can enhance or downregulate T-cell activation, and others which, in culture, can affect the differentiation, maturation and viability of Langerhans cells (Luger & Schwartz 1991), functions which it might well subserve in vivo. A reciprocal influence of the Langerhans cell on keratinocyte proliferation, differentiation, and keratinization is enshrined in the concept of the Epidermal Proliferative Unit (p. 387).

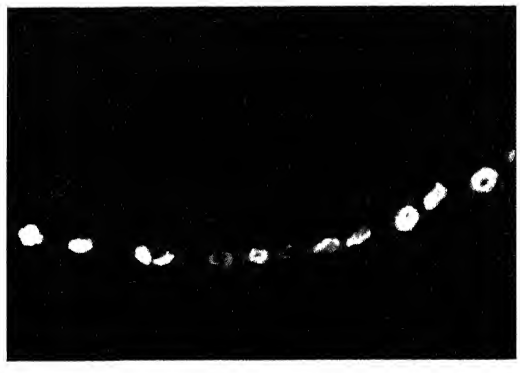
Langerhans cells are involved in rejection of skin grafts from histo-incompatible animals, and their absence from the cornea may be an important factor in the ease with which grafts of this tissue can be accomplished. Transplantation of bone-marrow cells from one individual to another is increasingly being used in the treatment of leukaemias and other blood discrasias, and in the process, precursor Langerhans cells will be transferred from donor to host and possibly involved, in addition to host Langerhans cells, in cutaneous manifestations of *graft-versus-host (GVH) disease* (Breathnach & Katz 1987). In acquired immunodeficiency syndrome (AIDS), epidermal Langerhans cells are greatly reduced in number, partly due to direct infection with the human immunodeficiency virus (HIV)-I, and partly due to the general state of immunosuppression.

**UV light and various chemicals.** Langerhans cells are adversely affected by these factors, which can inactivate them and deplete their numbers. In that they are normally involved in monitoring endogenous tumour antigens, this suppressive effect may contribute to the higher incidence of epidermal carcinomas and melanomas in caucasians habitually exposed to strong sunlight, and may play a part too in the chemical induction of neoplasms. Histiocytosis-X is a condition characterized by abnormal proliferation of cells morphologically practically identical with Langerhans cells, though differing somewhat in phenotype. Lesions occur in skin, lymph nodes, lungs and bone, and the condition is variously regarded as a tumour or a granuloma of Langerhans cells. For reviews, and citations of the voluminous literature on Langerhans cells and current unsolved problems which they present, see Schuler (1991), Bergstresser et al (1992), and Kamperdijk (1993).

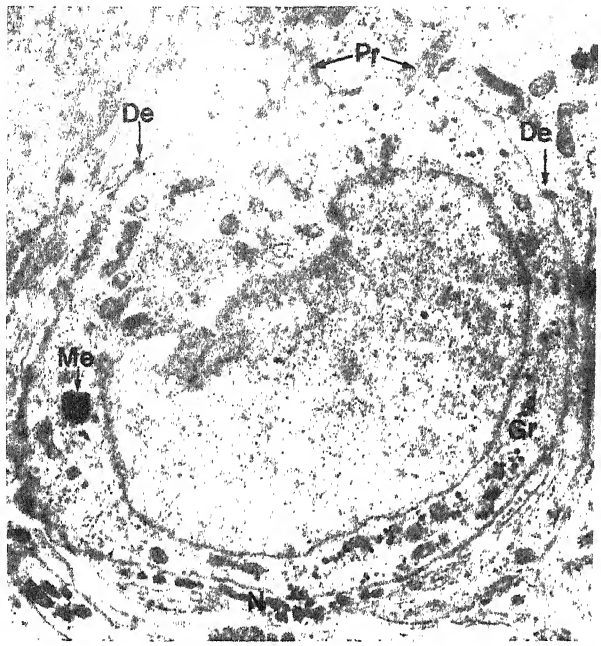
## Lymphocytes

Lymphocytes are occasionally seen in normal human epidermis, individually, or in apposition with Langerhans cells and melanocytes. Their presence is readily understandable in the context of SALT. Mast cells have also been reported in normal epidermis, though this raises the question of what is 'normal'. In present civilization the

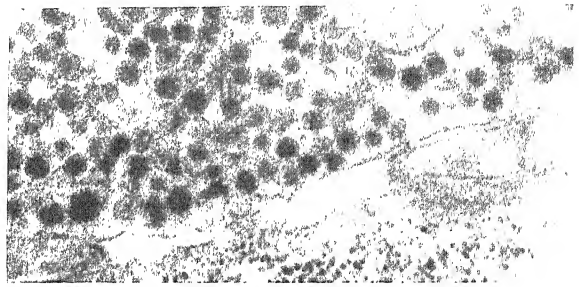


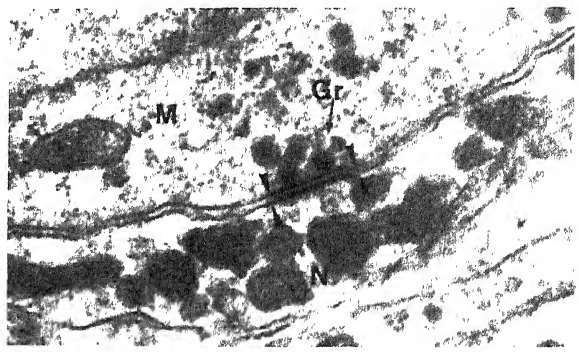

5.34 A, B. Immunolabelling of Merkel cells from rabbit lip. A shows a semithin section in which are 'clear cells' (arrowheads) in the basal layer of the epidermis, representing Merkel cells, as shown by later electron microscopy. B shows a similar section immunostained for the low molecular weight keratin 18 located in basally placed Merkel cells. Magnifications: A × 300, B × 280. (Provided by Professor J H Saurat, Dermatology Clinic, Cantonal Hospital of the University, Geneva, Switzerland.)




**5.35** A–C. Electron microscopic appearance of Merkel cells and related structures. A is a Merkel cell in the basal epidermal layer of a human fetal finger, showing general features. Note desmosomes (De) between the Merkel cell and adjacent keratinocytes, characteristic granules (gr), phagocytosed melanosomes (Me), nerve ending (N) in contact with the basal aspect, and spine-like processes (Pr) indenting the neighbouring keratinocytes. Magnification × 16 800. (From Winkelmann and Breathnach 1973, with permission.)



**5.35**B. Characteristic granules in a fetal Merkel cell, each granule consisting of a dense core separated by a lucent interval from the surrounding membrane. Magnification × 62 300. (Supplied by D Robins, Department of Anatomy, St Mary's Hospital Medical School, London. From Winkelmann and Breathnach 1973, with permission.)




**5.35**c. A region of apposition between a fetal Merkel cell (M) and an afferent nerve terminal (N). In the segments between the arrowheads the membrane shows increased density granular vesicles (Gr) are concentrated here, resembling a type of synapse in its appearance. Magnification × 70 200. (Reproduced by permission from Breathnach 1979.)

epidermis in different cultures is submitted to so many cosmetic and environmental assaults that it is difficult to establish what might be considered as virginal normal—perhaps only the neonatal.

## Merkel cell

Merkel cells are morphologically distinct cells, present in the epidermis of mammals, amphibians, and fish, and in other vertebrates. In man, they appear in routinely stained preparations as 'clear' oval cells, singly or in groups, in the basal layer of the epidermis, especially of glabrous skin, and intimately associated with a nerve terminal. They are also present in the outer root sheath of some large hair follicles. They can be distinguished from other 'clear cells' (melanocytes and Langerhans cells) by a variety of specific immunohistochemical reactions (**5.34**A, B). Clusters of Merkel cell–neurite complexes in glabrous skin are called *touch corpuscles* (*Tastscheiben*), and in hairy skin, *tactile hair discs* (*Haarscheiben*). Merkel cells are regarded by some as belonging to a widely-distributed system of 'neuroendocrine cells', also known as the '*amine precursor uptake and decarboxylation* (*APUD*) *system*' (Winkelmann 1977) and '*paraneurons*' (Fujita 1977).

**Ultrastructure** (see Halata 1975). Short, spiny processes of the plasma membrane with a cytoplasmic core of intermediate filaments insinuate between, or indent, adjacent basal keratinocytes, to which the Merkel cell is attached by small desmosomes (**5.35**A). The basal plasma membrane is closely apposed to the membrane of an axonal terminal packed with mitochondria and clear-cored vesicles, and areas of membrane specialization, seen as localized densities, may be present along the apposition (**5.35**c; see also **8.73**c). The cytoplasm contains common organelles, numerous closely-packed intermediate filaments, and characteristically, 80–110 μm dense-core granules (**5.35**B). These are usually concentrated mainly on the side of the nucleus opposite to the Golgi apparatus, from which they are thought to be derived, and may be closely associated with areas of basal plasma membrane specialization. The nucleus may contain nuclear rodlets, also present in some nerve cells.

**Immunoreactivity.** Merkel cells express the low molecular-weight cytokeratins 8, 18, and 19, but not the types characteristic of fully differentiated keratinocytes (Moll et al 1982). They also show immunoreactivity to the following antigens: neuron-specific enolase (NSE), vasoactive intestinal polypeptide (VIP), met-enkephalin, chromogranins, and bombasins (Hartschuh et al 1979; Hartschuh et al 1983; Gu et al 1981). Dense-core granule-containing neuroendocrine cells exhibit similar reactivities. Human fetal dermal Merkel cells have been reported as expressing nerve growth factor (NGF) receptors (Narisawa et al 1992).

**Nature and function.** The developmental lineage of Merkel cells is not yet fully established, although the view that they are modified keratinocytes (Moll et al 1986) is rapidly replacing the theory that they are immigrants which traverse the dermis along developing neurites (Breathnach 1979). In favour of the view that they are modified keratinocytes, or arise from a common ectodermal stem cell, is the observation that in development they appear in the epidermis (between weeks 8–12) earlier than in the dermis, preceding the ingrowth of nerve fibres (Moll et al 1986; Pasche et al 1990; McKenna Boot et al 1992). Human fetal skin (8–11 weeks), lacking Merkel cells and xenografted to nude mice, contained abundant epidermal Merkel cells of apparent human origin after 4–8 weeks (Moll et al 1990). This evidence suggests that fetal dermal Merkel cells are derived by migration from epidermis to dermis, and not vice versa, and that they become secondarily associated with nerve fibres. This is consistent with evidence that Merkel cells serve as targets for ingrowing axons during development, evoking directional sprouting of fibres and partially determining terminal fields (Diamond 1979).

Precise localization to Merkel cell–neurite complexes of mammals, of slowly adapting Type 1 discharges following mechanical stimulation of the skin, confirms that they are mechanoreceptors. They are capable of detecting vertical, shearing, or other directional deformations, and direction of hair movement. Morphology suggested that the Merkel cell was the prime transducer giving rise to a

receptor potential activating the associated axon by a synaptic mechanism involving release of a transmitter substance from the dense-core cytoplasmic granules. However, no transmitter substance has been detected in the granules, and electrophysiological evidence of chemosynaptic transmission from Merkel cell to nerve ending is lacking. A trophic function, or a function similar to that of the Schwann cell for the neurite has also been suggested for the Merkel cell. For further details of this cell, see page 967.

### Epidermal symbionts

A concept of the epidermis as a compound tissue made up of cellular elements of different developmental origins and prime functions (epidermal symbionts), existing together in biological balance and mutual dependence to perform wider collaborative functions, has grown out of early tentative ideas of Caudiere, Billingham, and Rappaport, and now includes the subconcepts of the Epidermal Melanin Unit, the Epidermal Proliferative Unit, and SALT (p. 1442). The symbionts consist of the keratinocytes, melanocytes, Langerhans cells, and lymphocytes. Whether the Merkel cell should be included is problematic. In the accounts above, many instances have been quoted of one cell type influencing proliferation, differentiation and functioning of others (sometimes facilitating, sometimes blocking activities), and being, in turn, affected similarly by these. Such interactions are of importance in development, growth, inflammation, immunology and wound-healing, and for maintaining normal homeostasis in the epidermis. They involve expression of cell-surface molecules—adhesion molecules, interleukins, various antigens, receptors—as well as growth factors, cytokines, and other factors involved in signalling, both intracellular and extracellular. For example, there is evidence that keratinocyte-derived cytokines regulate the functioning of Langerhans cells, and that Langerhans cell-derived cytokines in turn regulate the activities of keratinocytes. Identification of such substances and understanding their actions in health and disease is a major concern of current dermatological research. It has been pointed out that epidermal activities may be controlled and affected by extraepidermal influences, such as hormones, etc. and it is important to remember that substances produced within the epidermis, not only vitamin D, but also some of those mentioned above can, by diffusion or by entering the circulation, have wider local and systemic effects.

## DERMIS

The dermis (**5.1**, **36–38**) is an irregular, moderately dense, soft connective tissue, with a matrix composed of an interwoven collagenous and elastic network in an amorphous ground substance of glycosaminoglycans, glycoproteins, and bound water, which accommodates nerves, blood vessels, lymphatics, epidermal appendages and a changing population of cells. Mechanically, the dermis provides considerable strength to skin by virtue of the number and arrangement of its collagen fibres, which give it tensile strength, and it has elastic recoil because of its elastic fibres. The density of its fibre meshwork, and therefore its physical properties, varies both within an area and with different sites, and with age and sex. The dermis is vital for the survival of the epidermis, and important morphogenetic signals are exchanged at the interface between the two, the epidermal–dermal junction, or basement membrane zone (BMZ), during development and postnatally (see p. 297). The dermis can be divided into two zones, a narrow superficial *papillary layer*, and a deeper *reticular layer*, though the transition between the two is gradual.

### Dermal collagen

General aspects of biosynthesis, structure, and types of collagen are dealt with elsewhere (p. 81). Types I and III form the major part of adult dermal collagen in the proportions of 80–85% and 15–20% respectively. The coarser-fibred Type I is predominant in the reticular dermis, and the finer Type III is found in the papillary dermis and around blood vessels. Type IV collagen forms the lamina densa of the basal lamina (p. 397) where it provides a scaffold for interactions between cells and amorphous matrix components such as laminin and heparan sulphate proteoglycan; it is also present in the basal laminae of Schwann cells and endothelial cells, and in anchoring plaques. Type V occurs in the lamina lucida and sparsely around cells, and Type VI forms a microfibrillar network throughout the dermis, enmeshing nerves and vessels. Type VII is the main component of anchoring fibrils (**5.39**).

**5.36** Scanning electron micrograph of the surface of a section through the skin showing the epidermis (above) and the layers of the dermis. The papillary layer close to the epidermis contains fine collagen fibres, while in the deeper reticular layer the fibres become increasingly more coarse. Magnification ×300.

### Elastic fibres

For general aspects, see page 83. Elastic fibres form a fibrous network interwoven between the collagen bundles throughout the dermis (**5.38**). They consist of two components, amorphous elastin and 10 nm microfibrils. Close to the dermal–epidermal junction, only microfibrils known as *oxytalin* are present; somewhat deeper is *elaunin*, composed mainly of fibrils with little elastin, and deeper still are the *mature elastic fibres* with a predominance of elastin. They form a continuum extending deeper from the underside of the lamina densa.

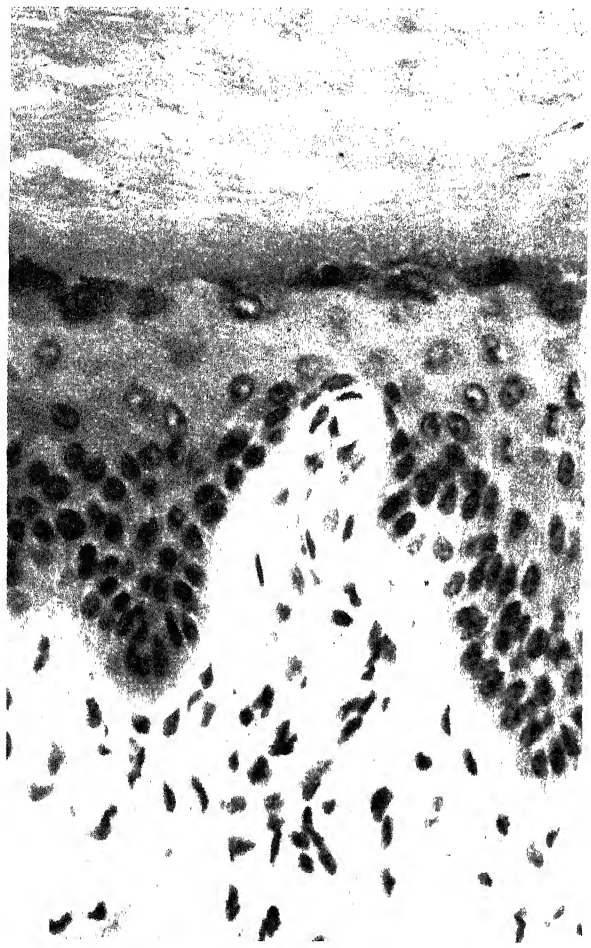
### Ground substance

For general aspects, see page 84. The ground substance of the dermis, being amorphous, can only be visualized by special techniques and consists mainly of proteoglycans (glycosaminoglycans being, predominantly, hyaluronic acid and dermatan sulphate, heparan sulphate, and some chondroitin-4 and chondroitin-6 sulphate) and fibronectins. These matrix macromolecules interact with cell surface and transmembrane molecules and maintain the cellular environment of the dermis, including its water and electrolyte composition. It is also concerned with cell movement and attachment to substratum during development and wound healing (see p. 412 et seq). For more on the extracellular matrix in general, see Hay (1981, 1982), Uitto et al (1989), and Alberts et al (1994).

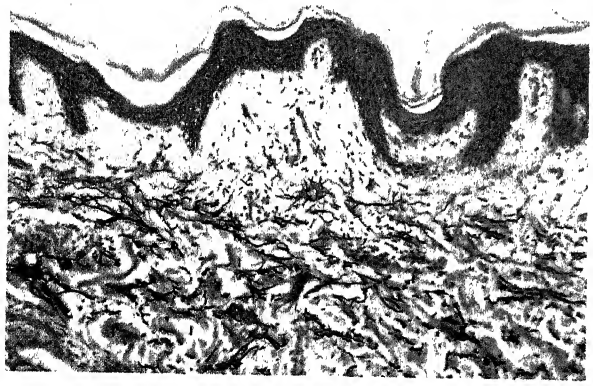
### Cells of the dermis

Two major categories of cells are present in postnatal dermis:

- fixed cells of organized structures such as nerves (Schwann cells, epi-, peri- and endoneurial cells), vessels (endothelial cells, pericytes, smooth muscle cells), cells of the arrector pili muscles
- a population of free cells of different origins and functions, the composition of which can vary with time and region so that it is not possible to define a normal distribution.



5.37 Vertical section through a dermal papilla and adjacent epidermis, showing a capillary loop. Notice the closeness of the vessel to the basal layer of the epidermis. Also visible are the layers of the epidermis, including a prominent stratum granulosum and above it the stratum lucidum stained dark orange and the paler orange stratum corneum. The section was taken from the thick skin of the foot. (Compare also 5.1 and 5.11.) Mallory's triple stain. Magnification × 800.

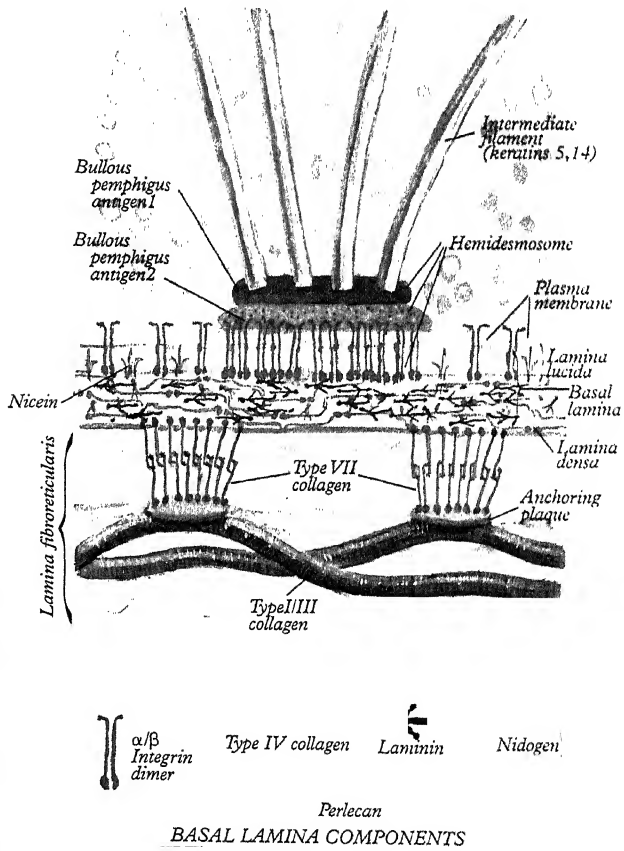


5.38 Section through thin skin, stained by the Verhoeff method to demonstrate elastin fibres. The fibres of the superficial layers of the dermis are thin while the deeper layers have more conspicuous coarser elastic fibres. Magnification × 120.

Among these latter cells are: fibroblasts, macrophages, mast cells, eosinophils, neutrophils, T-lymphocytes, and B-lymphocytes (including plasmacytes), and dermal Langerhans cells, all of which can be identified at light or electron microscopical level either by special staining techniques or by morphological characteristics including specific marker organelles, and whose functions are clear. Then, there are others, not easy to identify on any basis, but which have been referred to under such terms as fixed histiocytes, or monocytic cells. Headington (1986a,b) suggests the term *histiocyte* be abandoned, and identifies cells currently so described as *dermal dendrocytes* of bone-marrow derivation and capable of antigen presentation. He further suggests that many cells regarded as fibroblasts are, in fact, dermal dendrocytes. Sontheimer (1989) has described a 'dermal perivascular dendritic macrophage' expressing major histocompatibility complex (MHC) class II (HLA-DR) antigens which may engage in immunological reactions with microvascular endothelial cells, and perivascular T-lymphocytes. These observations indicate a greater participation of dermal elements in immunology than previously thought.

## Layers of the dermis

**Papillary layer.** This is immediately deep to the epidermis (5.3, 36, 37), and is specialized to provide mechanical anchorage, metabolic support, and trophic maintenance to the overlying tissue, as well as housing rich networks of sensory nerve endings and blood vessels. Its superficial surface is marked by numerous papillae which interdigitate with recesses in the base of the epidermis and form the dermal–epidermal junction at their interface. The papillae have round or




BASAL LAMINA COMPONENTS

5.39 Diagram showing the major features of the basement membrane zone of skin, including some of the important molecular species involved. See text for further details.

blunt apices which may be divided into several cusps. In thin skin, especially in regions with little mechanical stress and minimal sensitivity, papillae are few and very small, while in the thick skin of the palm and sole of the foot, they are much larger, closely aggregated, and arranged in curved parallel lines following the pattern of ridges and grooves typical of these surfaces (**5.1**). Lying under each epidermal ridge are two longitudinal rows of papillae one on either side of epidermal *rete pegs* through which the sweat ducts pass on the way to the surface (see **5.1, 3**). Each papilla contains narrow, densely interwoven bundles of fine Type I and III collagen fibres, some elastic fibrils and microfibrils, many attached to the basal lamina and extending deeper. Also present is a capillary loop, and in some sites, especially in thick hairless skin, Meissner's corpuscular nerve endings (p. 967).

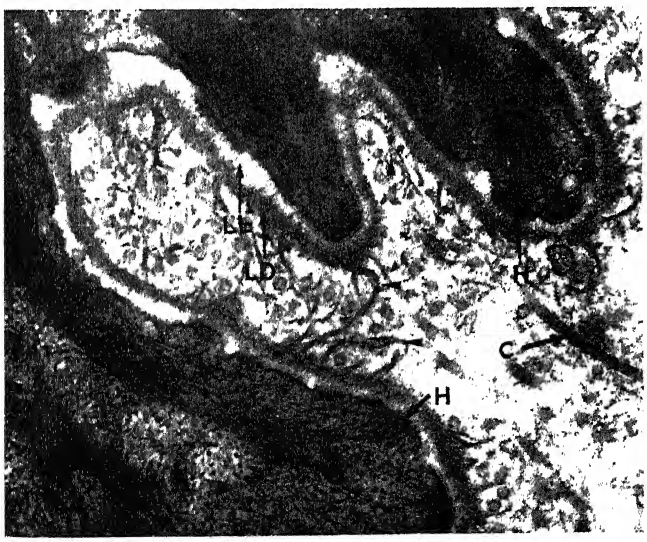
**Reticular layer.** This merges with the deep aspect of the papillary layer (**5.3, 36**). Its bundles of collagen fibres are thicker than those in the papillary layer and interlace with them and with each other to form a strong yet deformable three-dimensional lattice, in which many fibres are parallel to each other, and within which lies a variable number of elastic fibres. The predominant orientation of the collagen fibres may be related to the main direction of action of the mechanical forces to which the dermis is subjected locally and thus may be involved in the development of skin surface lines (p. 378).

## DERMAL–EPIDERMAL JUNCTION

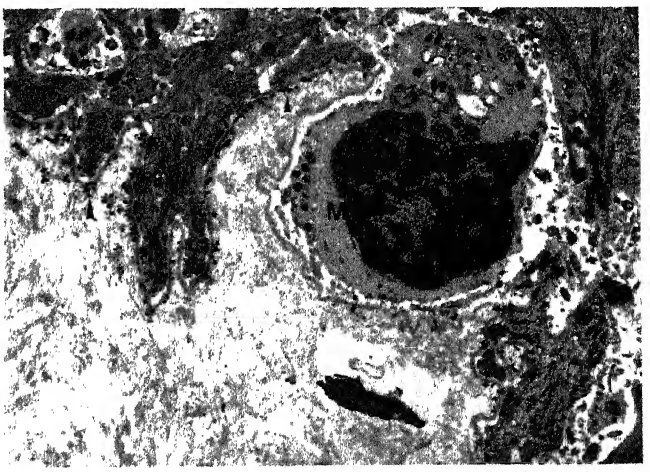
In sections perpendicular to the surface, the dermal–epidermal junction exhibits various degrees and patterns of undulation related to the prominence or otherwise of dermal papillae. Staining with periodic acid-Schiff (PAS) technique reveals an apparent *basement membrane* along the junctional line, and a similar appearance occurs in other situations where cellular structures interface with the extracellular matrix (see p. 80). However, electron microscopy reveals that there is no such structural entity, and it is more correct to refer to a *basement membrane zone* (BMZ), which in the skin consists of several components (**5.39–41**). There is the *basal cell membrane*, studded with hemidesmosomes, beneath which is the *basal lamina* consisting of an electron-lucent *lamina lucida* of 40–50 nm traversed by *anchoring filaments* which insert into an amorphous *lamina densa* of approximately 70 nm; this may be intermittently reduplicated. Beneath the basal lamina is a shallow *reticular layer* of different fibrous elements: banded *anchoring fibrils*, attached to the lamina densa at one end, and ending freely or looping back, or being attached to amorphous *anchoring plaques* at the other; oxytalin *microfibrils*; and small diameter *collagen fibrils*. Beneath melanocytes, anchoring filaments traverse the lamina lucida, but anchoring fibrils are practically absent beneath the lamina densa. The cytoskeleton of the epidermal keratinocyte is linked to the fibrous matrix of the dermis through the attachment of keratin filament bundles to hemidesmosomes, via anchoring filaments across the lamina lucida to the lamina densa, from which anchoring fibrils (Type VII collagen: **5.39**) and oxytalin microfibrils extend deeply. This arrangement provides a mechanically stable substratum for the epidermis. Epidermis and dermis can be separated, usually in the plane of the lamina lucida, by trypsinization or immersion of skin in various chemicals. This provides '*split-skin*' preparations which are useful for examining the apposed surfaces of the two en face, providing, for example, excellent views of the pattern of dermal papillae, and detailed ultrastructure of the lamina densa (Mihara et al 1992) by scanning electron microscopy, and an opportunity to carry out counts of melanocytes in DOPA-stained epidermal sheets. In inherited bullous (blistering) diseases of the BMZ, separation can occur within the cytoplasm of the basal cell, along the plane of the lamina lucida, or just beneath the lamina densa.

Many BMZ components are precisely localized. The lamina densa is mainly composed of Type IV collagen and the anchoring fibrils of Type VII collagen (**5.39**). *Laminin*, a glycoprotein which binds to cells and Type IV collagen, is present in the lamina densa and lucida, and *entactin nidogen*, which binds to the other three, is present in the lamina densa. *Heparan sulphate proteoglycan* (perlecan) and *chondroitin-6-sulphate proteoglycan* are present in the lamina densa, and *bullous pemphigoid antigen* is largely localized to hemidesmosomes. Other antigens characterized by monoclonal antibodies have been recognized. These various components are synthesized by epidermal keratinocytes and/or by dermal fibroblasts, and react with one another and with fibronectins in the formation of an organized BMZ during development, in its maintenance throughout postnatal life and in its reconstitution during wound-healing and re-epithelialization. Similar interactions are involved in facilitating cell movements (Langerhans cells, lymphocytes) in either direction across the dermal–epidermal junction.

Since the epidermis is non-vascular, clearly, macromolecules and solutes essential for the nutrition of the cells must pass the barrier of the basal lamina. The permeability properties of the lamina are, therefore, of interest and significance, but it is not easy to locate definitive information in the literature.




**5.40** Electron micrograph of a section through the base of the epidermis showing the epidermal–dermal junction. Note the basal keratinocyte plasma membrane with hemidesmosomes (H), lamina lucida (LL), lamina densa (LD), anchoring fibrils (arrowheads) attached to its deeper aspect and adjacent collagen fibrils (C). Magnification × 45 000. (Provided by J McGrath, St John's Dermatology Centre, UMDS, St Thomas' Campus, London.)




**5.41** Immunogold labelling of anchoring fibrils at the epidermal–dermal junction. Gold particles (arrowheads) are seen beneath the lamina densa underlying the keratinocytes, but are virtually absent from this situation beneath a melanocyte (M). Magnification × 4680. (Provided by J McGrath, St John's Dermatology Centre, UMDS, St Thomas' Campus, London.)

## INNERVATION OF SKIN

Skin is a major sensory surface, with regional variations in sensitivity to different stimuli evoking a spectrum of subjective sensations. It has a rich nerve supply, which is also concerned with autonomic functions, in particular, thermoregulation. Equating structure and distribution of fibres and receptors with function has been a major and continuing concern of neuroanatomists and neurophysiologists over the years.

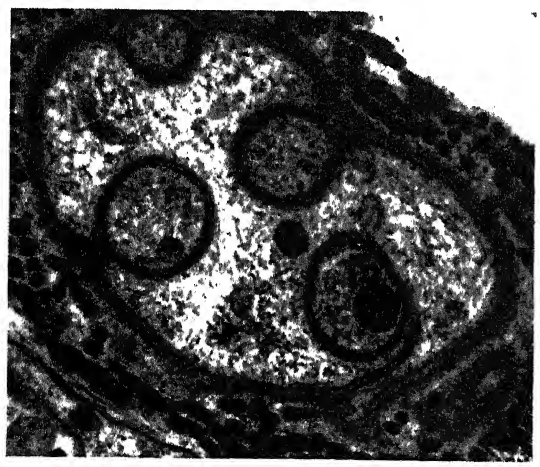
Cutaneous sense provides us with a wealth of information about the external environment and its interactions with the skin, through receptors tuned to stimuli of various kinds. These latter may be classed as: mechanical (rapid or sustained touch, pressure, vibration, stretching, bending of hairs, etc.), thermal (hot and cold), and noxious (perceived as discomfort, itching and pain of various degrees of intensity). In addition, there are other stimuli evoking sensations less easy to define precisely, such as those of pleasure evoked by appropriate stroking, tickle, or wetness. All of these sensations are recorded and interpreted by a wide variety of specialized neurons distributed throughout the ascending levels of the nervous system, but the primary input is transmitted by neurons whose cell bodies lie in the spinal and cranial ganglia, and whose fibres are terminally distributed to the dermis. These may be myelinated or unmyelinated (5.42).

Efferent autonomic fibres are non-myelinated noradrenergic and cholinergic in type, innervating the arterioles, arrector pili muscles, and the myoepitheliocytes of sudorific and apocrine glands. In the scrotum, labia minora, perineal skin and nipples they also supply smooth muscle fasciculi of the dermis and adjacent connective tissue. Except in the nipples and genital area, activity of the autonomic efferent nerves is chiefly concerned with regulation of heat loss by vasodilation and vasoconstriction, sweat production, and (only incipiently in humans) pilo-erection.

### Dermal plexuses, nerve terminals and receptors

On reaching the dermis, nerve fasciculi branch extensively to form a deep *reticular plexus*, which serves much of the dermis, including most sweat glands, hair follicles and the larger arterioles. Many small fasciculi pass from this plexus to ramify in another superficial *papillary plexus* at the junction between the reticular and papillary layers of the dermis. Twigs from this pass more superficially into the papillary layer, ramifying horizontally and vertically, terminating either in relation to encapsulated receptors, or as terminals reaching the level of the basal lamina, and, in some instances, entering the epidermis. As these latter fasciculi proceed superficially, the epineurial sheath merges with the general matrix collagen; the perineurium becomes reduced to a single cellular layer and eventually terminates, leaving Schwann-cell axonal complexes, enveloped by basal lamina, in direct contact with the matrix. Perineurial cells are joined by tight junctions, so the sheath forms an effective barrier against substances or organisms entering the endoneurial compartment across it. They can, of course, gain entry and proceed proximally from below the level at which it terminates (see also p. 947).

The detailed structure, classification, and behaviour of the sensory endings are described in detail in Nervous System (p. 962).



5.42 Unmyelinated neurite in papillary dermis consisting of Schwann cell process enveloping four axons. Note Schwann cell basal lamina, and sectioned collagen fibres. This is peripheral to the level at which the perineurial sheath terminates. Magnification × 39 375.

### Development of cutaneous nerves

Sensory cutaneous nerves—axons and Schwann cells—are derived by outgrowth from the neural crest (via posterior root ganglia), and motor fibres to vessels and glands arise from cells of sympathetic ganglia. As individual parts of the embryo grow, the nerves grow and lengthen with them. Small neurites are present superficially at a stage when the epidermis is bilaminar, and by 8 weeks' gestation there is already a functioning cutaneous plexus. By the fourth gestational month, the dermal plexuses are very richly developed, and by this time also, Meissner and Pacinian corpuscles appear. Whereas the overall general pattern of distribution of fibres is determined by forces intrinsic to the nervous system, the directional guidance of developing axons and the establishment of appropriate terminal connections is a very complicated matter and the subject of considerable differences of opinion amongst neuroembryologists (see p. 231). The growing tips of migrating axons have been much studied in tissue culture. The tip forms a swollen growth cone, the leading membrane of which may be flattened or ruffled, exhibiting filipodia, and it contains filaments, microtubules, and vesicles, all of which are associated with the axoplasmic transport which must accompany new membrane formation and elongation. GAP (Growth Associated Protein) 43, a protein which is a substrate for calcium-dependent and phospholipid dependent protein kinase C, influences the ability of axons to grow and is synthesized and transported to growth cones at a high concentration in extending axons. Swollen tips of axons can sometimes be observed in nerves of digital skin of human fetuses and possibly represent growth cones in vivo.

In the development of peripheral nerves, axonal outgrowth precedes migration of Schwann cells, so the latter do not serve as initial 'pathfinders'. The two soon become closely associated, however, to form the actual unit of migration, the nerve fibre whose progress is guided by local tissue conditions and influences. These are thought to include guidepost cells of other tissues, heterogeneities and gradients of matrix macromolecules along the way, and chemotropic factors released from target cells. They partly underlie the general overall processes of contact guidance and inhibition.

The ability of outgrowing neurites to adhere to cell surfaces they encounter plays a major role in directing their migration. Cell Adhesion Molecules (CAM, N-CAM) belonging to the immunoglobulin family (see p. 26) are involved, and integrins mediate adhesion between neurites and the matrix glycoproteins laminin and fibronectin. Homing of axons on to certain specific targets is thought to involve release of soluble diffusible factors, such as hormones and growth factors. Nerve Growth Factor (NGF) (Levi-Montalcini and Angeletti 1968) is produced by targets of sensitive adrenergic sympathetic neurons, such as sweat glands, and in vitro, has been shown to promote neurite outgrowth and adhesion of human sensory neurons in synergy with laminin and fibronectin (Crain et al 1980). Whether it functions in this manner in vivo has been questioned, but developing Merkel cells, which certainly serve as peripheral attractive targets for somatic terminals (Diamond 1979) have recently been reported to contain NGF (Munger 1991). It has been suggested that Epidermal Growth Factor may also be involved in directional guidance of axons during development. There is evidence that in development, terminal areas are approached by an excess of axons, and that there may be competition between them for specific targets or limited territories. Definition or limitation of final terminal distribution is governed partly by elimination of some axons, and excessive branching or extension is probably regulated by inhibitory mechanisms involving contact with neighbouring ones.

In embryos of 8–10 weeks, peripheral fascicles and their terminals consist of bundles of axons in direct contact, partially or completely enveloped by Schwann cells. Axons contain many microtubules which serve as a cytoskeleton, and are involved with axoplasmic

transport and motility. Schwann cells and processes soon invade the axonal bundles, separating them into progressively smaller ones, ultimately isolating them from one another, until finally a numerical relationship between cell and axon of one to one is established for myelinated fibres, and one to several for unmyelinated fibres.

These developments are associated with multiplication of Schwann cells, their migration in a transverse as well as a longitudinal direction, and degeneration of many axons. Axonal and other debris is frequently seen within fetal Schwann cells, and this potential for phagocytosis is carried forward into postnatal life.

The factors that determine myelination of an individual axon, and the final relationship between axon diameter, thickness of myelin sheath, and internodal length are matters of interest. It is generally agreed that the population of Schwann cells is uniform, and that size of axon (1 μm plus) is the trigger that signals the cell to start myelination. Axons with diameters of 1 μm undergoing myelination may be seen in human fetal cutaneous nerves as early as 12 weeks. Early during myelination, the Schwann cell expresses Myelin Associated Glycoprotein (MAG), an adhesion molecule which may be important for maintaining stability between it and the axon during initiation of the process. The total number of Schwann cells along an axon is fixed at the time of onset of myelination, as is the number of nodes of Ranvier (except in regenerating nerves).

Myelination involves the development of a multi-layered membranous sheath continuous with the Schwann cell plasma membrane. It is not a simple wrapping of pre-formed membrane around the axon, but the progressive addition of newly synthesized material at the inner and outer mesaxons, or at multiple sites. The lipid composition of myelin changes progressively during development, and is different to that of general Schwann cell plasma membrane (for further details, see p. 952).

The degree of myelination of peripheral nerves in the human fetus varies with the nerve, and the distance from the parent cell bodies at which it is examined. It is not possible therefore to give precise dates for the onset of myelination which would have general application. It can commence in the ulnar nerve as early as the twelfth week (Gamble 1964).

Early Schwann cell axonal complexes are surrounded by and in direct contact with the general mesenchymal collagen and matrix, and there is no collagen among the axons. The first sign of the connective tissue sheaths is the appearance of single cells loosely arranged around the neurites to form a primitive *perineurium*, and collagen fibres enclosed by them can be regarded as an early *endoneurium*. The primitive perineurial cells lack a basal lamina, but acquire one as the sheath becomes multilaminar; they are thought to be modified fibroblasts, and presumably produce the perineurial collagen. Endoneurial collagen is thought to be produced by both fibroblasts and Schwann cells, and the epineurium is formed by lamination of extra-perineurial collagen around the neurites. The outer lamellar cells of Pacinian corpuscles are homologous with the perineurium, but the source of the laminar cells of the Meissner corpuscle is unclear, in view of their direct contact with terminal axons, a relationship which is never seen with perineurial cells.

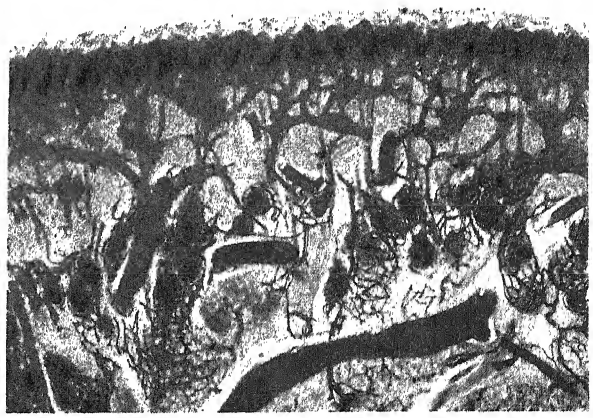
## VASCULARIZATION OF SKIN

### Blood vessels

The metabolic demands of the skin are not generally great, and yet, under normal conditions, the blood flow exceeds by 10 times its nutritional requirements, and may amount to 5% of the cardiac output. This is because the cutaneous circulation has an additional important thermoregulatory function, and is arranged so that its capacity can be rapidly altered by as much as 20 times in either direction in response to requirements of loss or conservation of heat.

Blood enters the skin from the underlying muscles and subcutis via small perforating arterioles which form an anastomosing horizontal *reticular plexus* (**5.1**, 43) at the interface between cutis and dermis. From this plexus, some arterioles pass deeply to supply the adipose tissue and, where present at this level, sweat glands and hair follicles. Other arterioles pass superficially, giving off anastomotic collaterals to glands and hair follicles, and form a second major horizontal plexus, at the junction of the reticular and papillary dermis, the *papillary plexus*. Capillaries from this plexus loop into the dermal papillae, usually one loop per papilla, and the loops drain into a *superficial venous plexus* intertwined with the arteriolar papillary plexus. This venous plexus in turn drains into a flat intermediate plexus in the reticular layer, which further drains into a deeper plexus, receiving from capillary beds surrounding glands and hair follicles, and closely associated with the arteriolar reticular plexus. This close association between arteriolar and venous plexuses permits exchange of heat between blood in vessels at different temperatures flowing in opposite directions, for example between cooler venous blood returning from the surface, and warmer arterial blood coming from the heart (counter-current heat exchange). This can allow for conservation or dissipation of heat, depending upon circumstances.

The general structure and arrangement of the microvasculature in general is dealt with in detail in another section (see p. 1465), so only features particular to skin will be considered here. In the deeper layers of the dermis, arteriovenous anastomoses are common, particularly in the extremities subject to cooling (hands, feet, ears, lips, nose), where, as *glomera* (see p. 1468) they are surrounded by thick muscular coats. Under autonomic vasomotor control, these vascular shunts, when relaxed, divert blood away from the superficial plexus and so reduce heat loss, while at the same time ensuring some deep cutaneous circulation and preventing anoxia of structures such as nerves which might otherwise be at risk. Extensive intercapillary anastomoses are also present. Generally, cutaneous blood flow is regulated and constantly adjusted according to the need for heat loss or retention, or also, in some areas of the body, according to emotional states. The normal vascular tone is a balance between neural (vasoconstrictor and vasodilator) and chemical influences affecting the musculature of the arterioles. In very cold conditions, the peripheral circulation is greatly reduced by vasoconstriction, but intermittent spontaneous intervals of vasodilatation result in recurring increases in temperature which prevent cooling to the level at which frostbite might occur (the hunting reaction). This is thought to be due to a direct effect of oxygen lack on the arteriolar constrictor muscle, rather than to a neural influence. The deeper dermal arterioles contain elastic tissue in the wall, and are surrounded by several layers of smooth muscle cells. More superficially, the muscle forms two layers, an inner longitudinal and an outer spiral one, and just before the capillary loop, individual myocytes or pericytes form an incomplete layer outside the endothelium. The postcapillary venules have one or two layers of contractile pericytes which can produce gaps between the endothelial cells and allow extravasation of fluid (Braverman 1989). Tight junctions are prominent between smooth muscle cells, pericytes, and endothelial cells, an arrangement which provides strength and stability to the vessel wall. For more on thermoregulation, see Clarke and Edholm (1993).



**5.43** A thick vertical section through palmar skin, the arteries, arterioles and capillaries of which have been injected with red gelatin to demonstrate the pattern of dermal vascularization. At the base of the dermis a broad flat arterial plexus supplies a more superficial papillary plexus, which in turn gives off capillary loops which enter the dermal papillae. Sweat glands and their ducts are numerous in this specimen; they extend basally into the subcutaneous tissues. Magnification × 200.

Individual segments of the dermal microvasculature can be identified on the basis of level, diameter, and more precisely, the components of the vessel wall (Braverman & Yen 1977; Higgins & Eady 1981). The *capillary loop* arises from a *terminal arteriole*, and ends in a *postcapillary venule*. At the arterial end, the ascending limb consists of an endothelial tube surrounded by a homogeneous basal lamina, outside which are individual pericytes, also enveloped by basal lamina. Just beyond the apex of the loop, the basal lamina becomes duplicated, and in the postcapillary venule it is multilaminated. Pericytes are more numerous in association with venules. Mast cells, and elongated fibroblast-like cells, or *veil cells*, are frequently seen closely associated with terminal arterioles and postcapillary venules. Sontheimer (1989) has defined a human *dermal microvascular unit* comprising the endothelial tube, pericytes, mast cells, and T-lymphocytes, and another cell, the *perivascular dendritic macrophage*, with antigen-presenting capabilities. It is probable that this cell belongs to the population of dermal dendrocytes (see p. 1415), and it may be identical with the veil cell. Sontheimer suggests the dermal microvascular unit may be a significant site of functional, immunological and other types of interaction between its cellular components.

The *endothelial cell* of the dermal microvasculature is particularly rich in microfilaments, which serve cytoskeletal and possibly contractile functions, and *Weibel-Palade bodies*, which store Factor VIII, are most numerous in the endothelium of venules. Fenestrated endothelial cells are present mainly in the capillary loops, and in association with vessels supplying the skin appendages. The endothelium must not be regarded merely as a passive, semi-permeable lining of the vessels. It has potential contractile and migratory abilities which become manifest in inflammatory and reparative processes, and expresses a large number of antigens, including Factor VIII-related antigen, and Class II major histocompatibility complex (MHC) antigens, and synthesizes cytokines, adhesion molecules and the angiotensin-converting enzyme (ACE) (Ruiter et al 1989). These synthetic properties are important in the endothelium's interactions with vasoactive amines of mast cells and nerves (Tharp 1989), in lymphocyte adhesion and migration, and in the recruitment of inflammatory cells into the skin. It is, therefore, a key cell involved in inflammatory and immunological reactions, and in wound-healing and repair.

### Lymphatics

The general features and topographical arrangement of the body's lymphatic system are dealt with elsewhere (see p. 1605). The lymphatics of the skin are small terminal vessels that collect fluid and macromolecules that have leaked from the capillaries for return to the circulation via larger vessels; they also convey lymphocytes, Langerhans cells and macrophages involved in immunological reactions to and from the regional lymph nodes. They begin as blind endothelial-lined tubes or loops just below the papillary dermis, which drain into a *superficial plexus* below the subpapillary venous plexus. This plexus drains via collecting vessels into a deeper plexus at the junction of the reticular dermis and subcutis, which in turn drains into the larger subcutaneous channels.

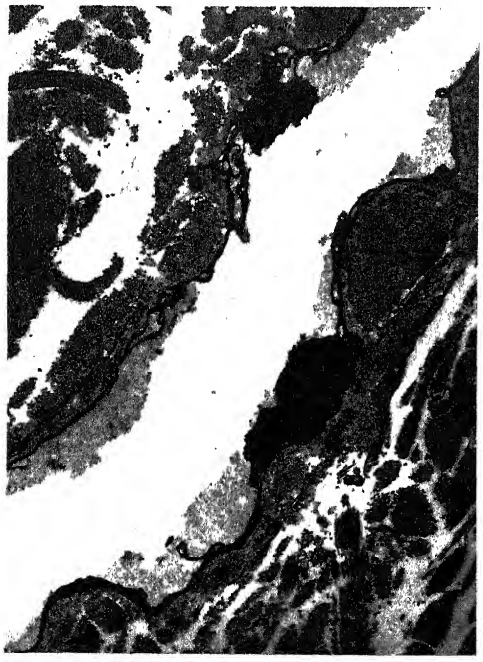
The wall of the terminal lymphatic vessel is formed by a single layer of very flattened overlapping endothelial cells, with few cytoplasmic organelles, and a tenuous, discontinuous basal lamina (**5.44**). Gaps of varying extent are present between the cells (**5.44B**), and protrusion into the lumen of cytoplasmic processes, nuclei and the tips of the inner of two overlapping cells gives rise to the appearance of *valves*. The endothelium of the larger collecting vessels is thicker, and the cells are connected by simple specialized junctional areas (p. 1605). Cells, macromolecules and fluid enter the lymphatics through the gaps in the wall, being directed thereto by 'wringing of the tissues' through movement of limbs, contraction of muscles and pulsation of adjacent arterial vessels (Ryan 1989, 1991). Unidirectional flow within the vessels is facilitated by the valves (Daroczy 1988).

## PILOSEBACEOUS UNIT

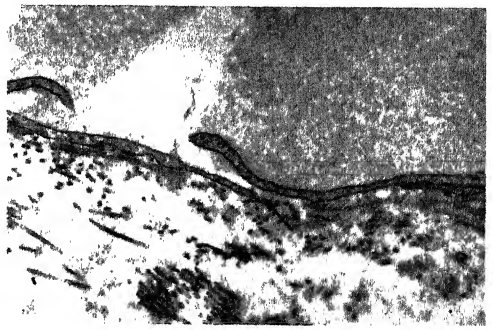
This comprises the hair and its follicle with associated arrector pili muscle, sebaceous gland, and sometimes an apocrine gland (**5.1, 45, 47**). Not all elements of the unit occur together in all bodily regions.

### Hairs

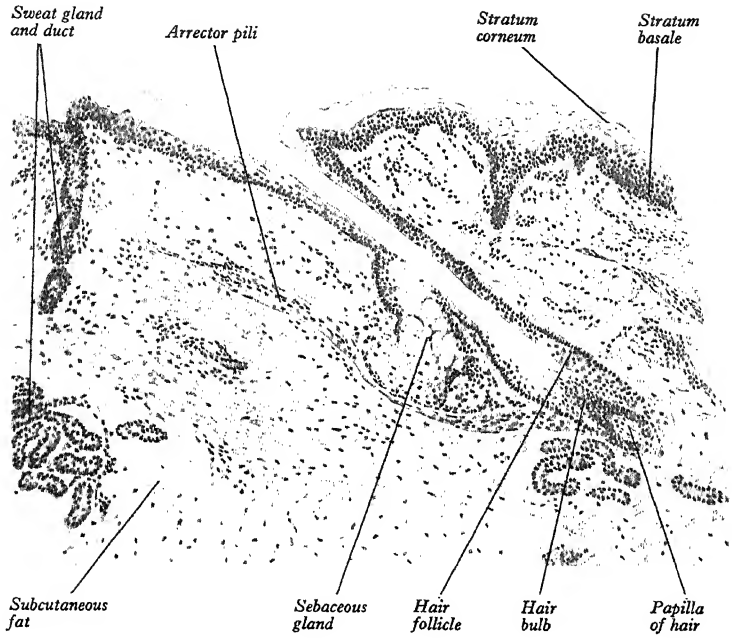
Hairs are filamentous keratinized structures present over almost all of the body surface, though less apparent in man than in his mammalian and primate ancestors. This 'trend towards nudity' in human evolution has been explained on a number of bases by anthropologists, including selective advantage on descending from the trees and hunting in a warm climate (Ebling 1991). The phylogeny of hair has also given rise to much speculation. Hair is thought to have developed in association with scales of pro-mammals, a 'basic trio-group' of three hairs being associated with a scale. Hairs grow out of the skin at a slant, and in small mammals are streamlined mainly in a craniocaudal direction. In many species this basic arrangement is complicated by reversals of orientation, with the formation of divergent streams and whorls to form '*hair-tracts*', the disposition of which is thought to be related to grooming habits,




**5.44A.** Lymphatic from papillary dermis. The vessel is lined by a layer of flattened cytoplasm of endothelial cells the nuclei of which project into the lumen. A basal lamina is not very evident and is discontinuous. Magnification ×3840.




**5.44B.** Portion of wall of terminal lymphatic from papillary dermis. A gap (arrow) is present at the overlapping edges of the lining endothelial cells, and the tip of the inner cell projects into the lumen. Magnification ×10 400.




5.45 A section through the skin, showing the epidermis and dermis (corium), a hair in its follicle, an arrector pili muscle and sebaceous glands opening into the hair follicle. Magnification × 100.

posture, or movement (Clark 1939). Hair-tracts can be mapped on the skin of man (Wiedersheim 1895), and the original streamlining is still evident in the sloping of the hairs on the dorsum of forearm, hand and fingers towards the ulnar side. This feature allows an isolated middle finger to be correctly sided at first glance. Hairs are absent from a few areas of the body, including the thick skin of palms, soles and flexor surfaces of the digits and certain other regions: umbilicus, nipples, glans penis and clitoris, the labia minora and the inner aspects of the labia majora and prepuce. The presence or absence, distribution and relative abundance of hair in certain regions (face, scalp, pubis, axillae) are secondary sexual characteristics which play subtle roles in sociosexual communication, and there are also racial variations in density, form, distribution and pigmentation. Within these parameters, there are also individual variations. Hairs assist minimally in thermoregulation, on the scalp they provide some protection against injury and the harmful effects of excessive solar radiation, and generally, they have sensory functions.

Hairs vary from about 600 per $cm^2$ on the face to 60 per $cm^2$ on the rest of the body. In length they range from less than a millimetre to more than a metre, and in width from 0.005 to 0.06 mm. They vary in form, being straight, coiled, helical or wavy, and differ in colour depending on the type and degree of pigmentation. Curly hairs tend to have a flattened cross-section, and are weaker than straight hairs. In general, body hairs are longest and coarsest in caucasians and least noticeable in mongolian races. Over most of the body surface hairs are short and narrow (*vellus hairs*) and in some areas these hairs do not project beyond their follicles, for example in eyelid skin. In other regions they are longer, thicker and often heavily pigmented (*terminal hairs*); these include the hairs of the scalp, the eyelashes, eyebrows and the postpubertal skin of the axillae and pubis, and the moustache, beard and chest hairs of males.

## Hair follicle

The hair follicle (**5.1, 45–50**) is an invagination of the epidermis (see p. 377) containing a hair, which may extend deeply (3 mm) into the hypodermis, or may be more superficial (1 mm) within the dermis. Typically, the long axis of the follicle is oblique to the skin surface; with curly hairs it is also curved. There are *cycles of hair growth* and hair loss, during which the follicle presents different appearances. In the *anagen phase* the hair is actively growing and the follicle is at its maximum development; this is followed by the involuting or *catagen phase* when hair growth ceases and the follicle shrinks; next comes the *resting* or *telogen phase*, during which the inferior segment of the follicle is absent. The next succeeding anagen phase follows. Further details of the hair growth cycle are given below, following the description of the anagen follicle and hair.

**Anagen follicle.** This has several regions. Deepest is the *inferior segment* including the region enclosing the *hair bulb* (**5.46, 47**) which extends up to the level of attachment of the arrector pili muscle at the *bulge*. Between this and the site of entry of the sebaceous duct is the *isthmus*, and above this level is the *infundibulum*, or *dermal pilary canal*, which is continuous with the *intraepidermal pilary canal*. Below the sebaceous duct, hair filament and follicular wall are intimately connected, and it is only towards the upper end of the isthmus that the hair becomes free in the pilary canal. Below the infundibulum the follicle is surrounded by a thick perifollicular dermal coat containing Type III collagen, elastin, sensory nerve fibres and blood vessels, and into which blend the arrector pili muscles. Marking the interface between dermis and follicular epithelium is a broad modification of the basal lamina, the *glassy membrane*.

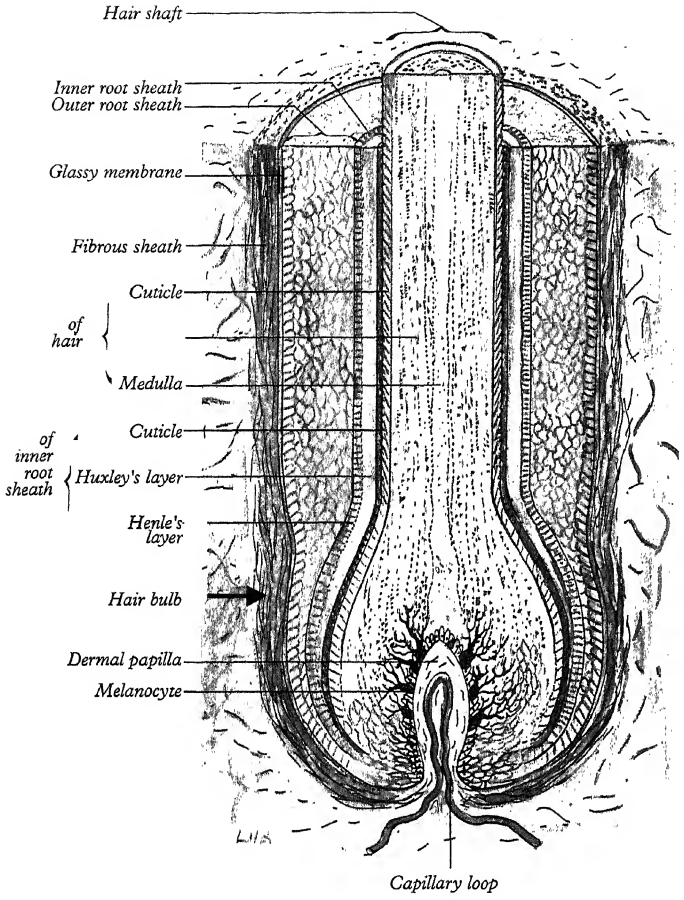
**Hair bulb.** This forms the lowermost portion of the follicular epithelium and encloses the *dermal papilla* of connective tissue cells (**5.47**). It generates the hair and its inner root sheath. A line drawn across the widest part of the hair bulb, or 'critical level', divides it into:

- a lower *germinal matrix*, of closely packed, mitotically active pluripotential keratinocytes, among which are interspersed melanocytes, and some Langerhans cells
- the '*upper bulb*' of cells arising from the matrix.

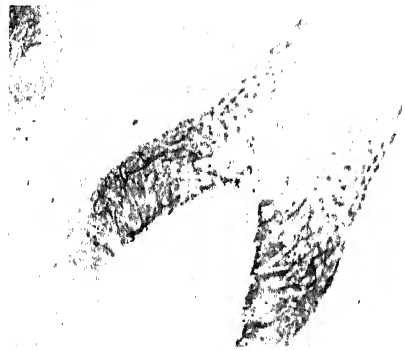
These latter move apically and differentiate along several lines. Those arising centrally form the hair *medulla*, then, radially, further out, successive concentric rings of cells will give rise to the *cortex* and *cuticle* of the hair and, outside this, the layers of the *inner root sheath*, from within out: the *cuticle of the inner root sheath*, *Huxley's layer* and *Henle's layer*. Outside Henle's layer is a layer of cells, the *outer root sheath*, which forms the wall of the follicle (**5.46, 49, 50**).

## Differentiation and structure of the hair and its sheaths

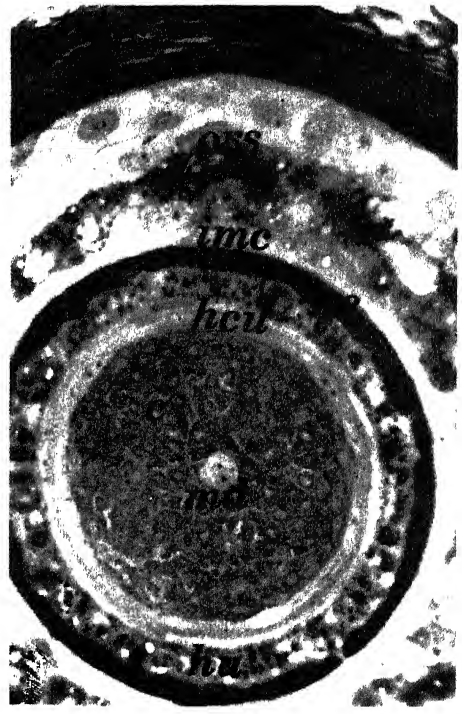
Differentiation towards keratinization of cells of the various layers of the hair and its inner root sheath commences at the level of the




5.46 Diagram illustrating the major structural features of the base of a hair follicle, showing the organization of the major layers of the hair and surrounding sheath, arising from the hair bulb. A dermal papilla invaginates the bulb, and along the interface between the dermis and epidermis, melanocytes insert their dendrites among the keratinocytes forming the hair.



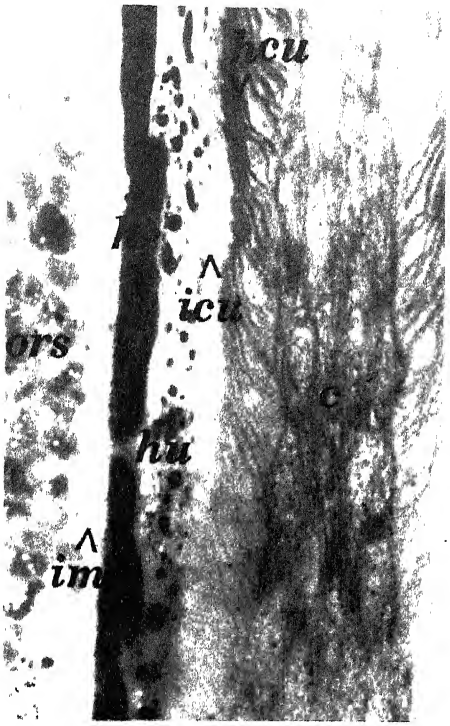
5.47 Vertical section through a hair root, showing the dermal papilla and numerous melanocyte processes extending into the matrix of the hair. Haematoxylin and eosin. Magnification × 250.



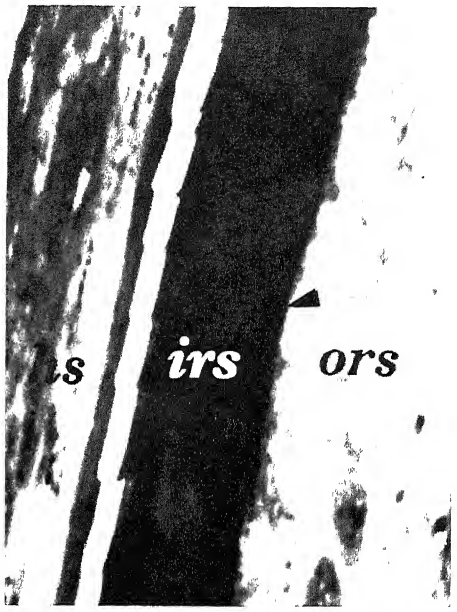

5.48 Transverse section of normal scalp anagen hair of a male aged 25, above the bulb and at a level at which only Henle's layer is keratinized. From without in are: connective tissue sheath (cts), outer root sheath (ors), innermost layer of outer root sheath (imc), keratinized Henle's layer (he), non-keratinized Huxley's layer with keratohyalin granules (hu), cuticles (hcu), outer lightly stained cuticle of inner root sheath and inner cuticle of hair, cortex (c), and medulla (md). Magnification ×525. (Reproduced from Tobin 1991, with permission.)

upper bulb and is asynchronous, beginning earliest in Henle's layer and Huxley's layer. It involves various morphogenetic and biochemical processes in which different cell migration patterns, cell shapes and distinct chemical forms of keratin are produced, depending upon which genes are being expressed. These processes are too extensive and complicated to be detailed here, but for morphological aspects, see Birbeck and Mercer (1957), Parakkal and Matoltsy (1964), Puccinelli et al (1967), Breathnach (1971), Montagna et al (1992), and for reviews of the biochemistry of hair keratinization see Baden (1990) and Gillespie (1991). An impression of the morphological transformations that take place is given by the illustrations in **5.46, 49, 55**. Excellent reviews of fundamentals of hair biology are provided in Wuepper et al (1993).

**Mature hair shaft** (**5.48–50**). This shows three concentric zones from outwards in, the *cuticle*, *cortex* and *medulla*, each with different types of keratin. In thinner hairs the medulla is usually absent. The *cuticle* forms the hair surface and consists of several layers of overlapping keratinized squames directed apically and slightly outwards (**5.51, 52**). In the isthmus region cells of the outer layer interlock with those of the cuticle of the inner root sheath (**5.50**). Pre-keratinizing cuticle cells have dense amorphous granules aligned predominantly along the outer plasma membrane with a few fila-

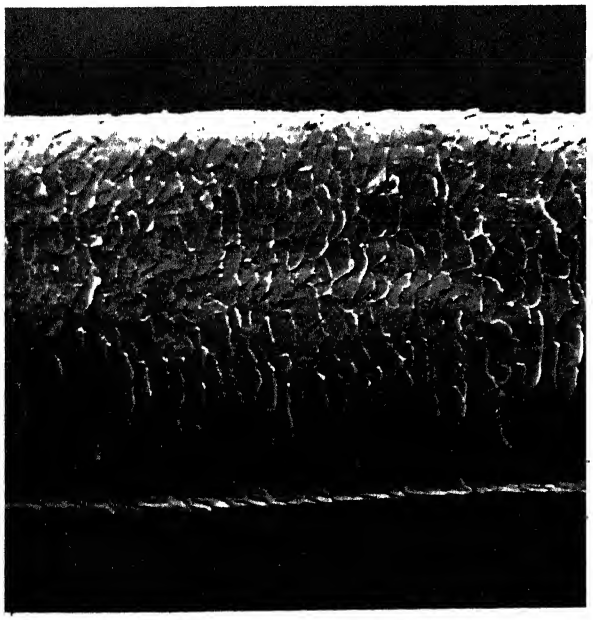



5.49 Longitudinal section of normal scalp anagen hair of a male aged 25 at a level at which only Henle's layer is keratinized. From without in are: outer root sheath (ors), innermost layer of outer root sheath (im), keratinized Henle's layer (he), non-keratinized Huxley's layer with keratohyalin granules (hu), cuticle of inner root sheath (icu) cuticle of hair (hcu), and cortex (c). Magnification × 1275. (Reproduced from Tobin 1991, with permission.)



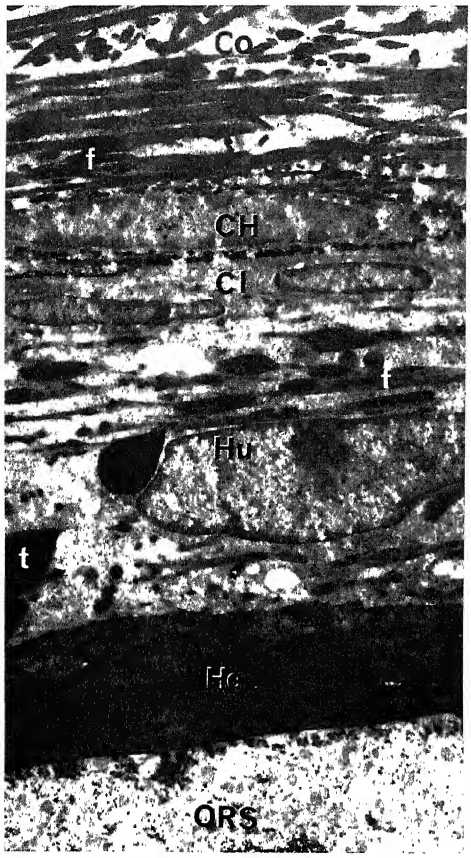

5.50 Longitudinal section of normal anagen scalp hair of a male aged 25 at a level below the entry of the sebaceous duct, and at which the hair and its inner root sheath are keratinized. From without in are: outer root sheath (ors), keratinized inner root sheath (Henle's (arrowhead) and Huxley's layers) and cuticle of inner root sheath (irs) and hair shaft (hs) consisting of narrow more deeply staining cuticle of hair and cortex. Note imbrication of apposed surfaces of cuticle of inner root sheath and cuticle of hair (bounding the artefactual space between them) which leads to interlocking. Magnification × 960. (Reproduced from Tobin 1991, with permission.)

ments, and when keratinized exhibit outer and inner zones of different densities, with a narrow dense band separating the cells (5.53). The cortex forms the greater part of the hair shaft and consists of numerous closely packed elongated keratinized cells which may contain nuclear remnants and melanosomes, and which are also separated by a narrow band of dense intercellular material. Pre-keratinizing cortical cells contain bundles of closely packed filaments but no dense granules, and when fully keratinized, exhibit a charac-



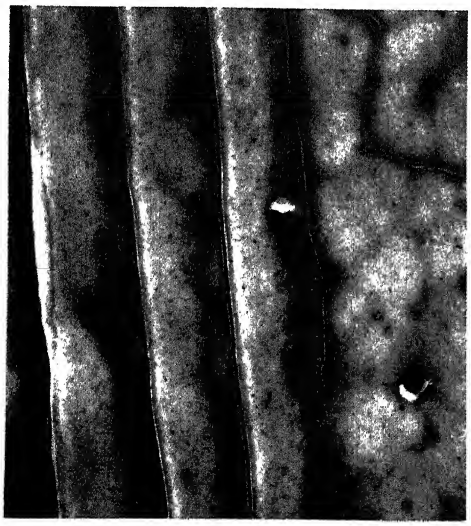
5.51 Scanning electron micrograph of a scalp hair showing details of surface structure. Note that the cuticular cells overlap each other; their free ends point towards the apex of the hair. Magnification × 370. (Specimen prepared by Michael Crowder, Guy's Hospital Medical School, London.)

5.52 Detail of 5.51. Magnification × 1850. (Specimen prepared by Michael Crowder, Guy's Hospital Medical School, London.)

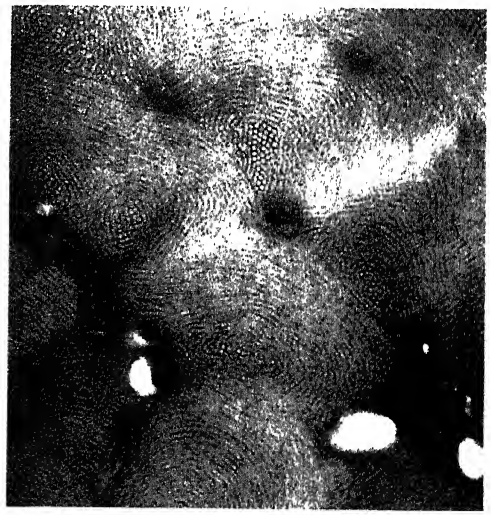



5.53 Longitudinal section of suprabulbar region of scalp hair of a 16½ week fetus. Direction of growth is towards the right. Note from without in: outer root sheath (ORS), keratinized Henle's layer (He), Huxley's layer (Hu) with trichohyalin granules (t) and filaments (f), cuticle of inner root sheath (Cl), cuticle of hair (CH), and cortex (Co) with dense filamentous bundles (f). With the exception of the outer root sheath, all the layers will ultimately become keratinized from differently arranged starting materials. Magnification × 5600. (Reproduced from Breathnach 1971, with permission.)

5.54 Pre-keratinized cuticles of adult hair. Below, the cuticle of the inner root sheath contains scattered round trichohyalin granules (t) and filaments, and desmosomes (d) are prominent along apposed plasma membranes. At the upper end of the field five to six layers of cells of the cuticle of the hair show characteristic dense granules aligned predominantly along the outer plasma membranes of the flattened cells. Magnification × 17 160. (Reproduced from Breathnach 1971, with permission.)



5.55A Keratinized cuticle and cortex of adult hair. The interior of the flattened cells of the hair cuticle, on the left, exhibit two zones of amorphous material, an inner dense, and an outer more translucent one; a narrow linear dense material occupies the intercellular space. The cortex, on the right, appears to have an amorphous structure also. At higher magnification it is seen to have a filamentous substructure. Magnification × 31 200. (Reproduced from Breathnach 1971, with permission.)



5.55B. Keratinized hair cortex, transverse section. The pattern is of axially orientated groups of electron-lucent filaments set in an amorphous matrix, and arranged in whorls to give the characteristic 'thumb-print' appearance. Dense material occupies the narrow intercellular intervals. Magnification × 8700. (Reproduced from Breathnach 1971, with permission.)

teristic 'thumb-print' appearance of electron-lucent filaments arranged axially and in whorls and set in a dense matrix (5.55). The medulla, when present, is composed of loosely aggregated and often discontinuous columns of partially disintegrated cells containing vacuoles, scattered filaments, granular material and melanosomes. Air cavities lie between the cells or even within them.

**Inner root sheath.** As already noted, this consists of three concentric layers of cells: the outermost two of which, *Henle layer*, and *Huxley's layer*, in the prekeratinized state contain irregular dense keratohyalin granules and associated filaments. At the level of the upper bulb Henle's layer begins to keratinize, as does Huxley's layer at the middle of the follicle, and when fully keratinized, cells of both have an apparently thickened envelope enclosing a filamento-amorphous matrix which undergoes some further change as they ascend. The cells of the *cuticle of the inner root sheath* have essentially the same structural components as the other two layers, though at the pre-keratinized stage the trichohyalin granules are much fewer, smaller, and rounded. Full keratinization takes place at a level lower than that of Huxley's layer, and, as with the other two layers of the sheath, before disintegration, the filamentous substructure is no longer apparent and a clear-cut pattern such as is seen in the cortical cells is not evident. As they become keratinized, the cells of the cuticles of the inner root sheath and hair become interlocked (5.49). Just below the level of entry of the sebaceous duct, the inner root sheath undergoes fragmentation, and the hair then lies free in the pilary canal.

**Outer root sheath.** Situated at the level of the upper bulb, this is a single or double layer of undifferentiated cells containing glycogen, which higher up the follicle becomes multilayered and gradually assumes the main characteristics of interfollicular epidermis, with which it becomes continuous. Langerhans cells and melanocytes are interspersed among its cells. At the level of entry of the sebaceous duct, it forms the wall of the pilary canal. In the fetus, the innermost cells contain keratohyalin granules and membrane-coating granules, and become keratinized, but this is less evident postnatally (Breathnach 1971). Ito (1989) describes two layers of cells in the outer root sheath with different keratin and antigen expressions during differentiation. This, and the suggestion that stem cells may reside in the sheath in the region of the bulge (see below), have directed attention towards a more active role of the outer root sheath than previously contemplated.

**Dermal papilla.** In the anagen follicle this consists mainly of highly cellular (fibroblastic) connective tissue continuous with the outer dermal sheath. They may be partially ensheathed by basal laminar material, and specialized contacts may be present where they are apposed (Tobin 1991). Macrophages and the occasional melanocyte may be present. During development, the dermal papilla induces formation of the hair germ, and is essential for maintenance of the follicle during ontogeny and postnatally through epidermal–dermal interactions similar to those operating at the basement membrane zone of interfollicular epidermis (see p. 297).

**Pigmentation of hair.** Melanocytes are present in the bulb in the region adjacent to the apex of the dermal papilla (5.47) and feed melanosomes to the medulla and cortex mainly. They are active only in mid-anagen, and during telogen become amelanotic, and are interspersed among the epithelial cells at the base of the club hair, where they can only be identified ultrastructurally. They are capable of producing both phaeo- and eumelanosomes, and changes in hair colour of an individual, usually in adolescence, are due to alterations in the dominant type produced. Greying of hair is due to a decline in numbers of melanocytes and their activities. In albinism, melanocytes are present in the bulb, but inactive.

## Hair cycle and growth of hair

Recurrent cyclic activity of hair follicles involving growth, rest, and loss of hair (moulting) in phases are characteristic of the pelage of mammals generally, but in man the cycles are irregular, of variable duration, with regional and other variations in the length of the individual phases. In the growing or *anagen phase*, follicle and hair are as described above. This is followed by the involuting or *catagen phase* during which mitotic activity of the germinative matrix ceases, the base of the hair keratinizes into a *club* which moves upwards to the level of the arrector pili muscle, and the whole inferior segment of the follicle degenerates; the dermal papilla also ascends and remains close to the base of the shortened follicle and its enclosed club hair. This situation persists during the resting or *telogen phase*. At the beginning of the next anagen, the epithelial cells of the base of the follicle renew mitotic activity to form a *secondary hair germ* which envelops the dermal papilla to form a new hair bulb. This grows downwards, reforming the inferior segment of the follicle, and from this a new hair grows up alongside the club hair (which is eventually shed). Cotsarelis et al (1990) have identified in mice a population of outer root sheath cells in the region of the bulb which they class as stem cells capable of regenerating the lower end of the follicle, an observation which calls into question the predominant or unique role of the matrix cells in this respect. (See also Lauer et al in Wuepper et al (1993) on this point.)

By the fifth month of fetal life the body is covered by fine, often deeply pigmented primary hairs all in anagen (collectively the *lanugo*); on the back these hairs are more frequent than those of the gorilla or chimpanzee at a similar age. Before birth some hairs may have reached catagen or telogen. Lanugal hairs are mostly shed before birth or immediately after, and are replaced by secondary *vellus hairs*, except on the scalp, eyebrows and palpebral margins. Since no further follicles are formed after birth, hairs become more widely spaced as the area of skin increases with body growth. Postnatally in man hairs exhibit regional asynchrony of cycle duration and phase leading to an irregular *mosaic pattern* of growth and replacement, as distinct from the *wave pattern* of rodents. In some regions the cycle is measured in years. Circannual variation of both scalp and thigh hair growth and loss in men occurs (Randall & Ebling 1991), with a greater number of anagen follicles on the scalp in winter, and a corresponding peak in the number shed during summer. This may represent a phylogenetic or evolutionary echo.

With puberty, hair growth and generation of much thicker hairs occurs on the pubes and axillae in both sexes, and on the face and trunk in males. The actions of hormones on hair growth are complex, and include not only sex hormones, but also those of the thyroid, adrenal cortex, pituitary and pineal (Ebling et al 1991). Androgens stimulate facial and general body hair formation and, after about the first 30 years, tend to cause the thick terminal hairs of the scalp to change to small vellus hairs, causing recession from the forehead and maybe almost complete baldness (*male pattern*). In females, oestrogens tend to maintain vellus hairs in their formation of minute hairs, and in postmenopausal life reduction of oestrogens may permit stronger facial and bodily hair growth. During midpregnancy, hair growth may be particularly active but some weeks later an unusually large number of hairs tend to enter the telogen phase and may be shed before the growth cycle recommences. In older men, growth of hairs on the eyebrows and within the nostrils and external ear canals increases, whereas elsewhere on the body growth slows and the hairs become much finer.

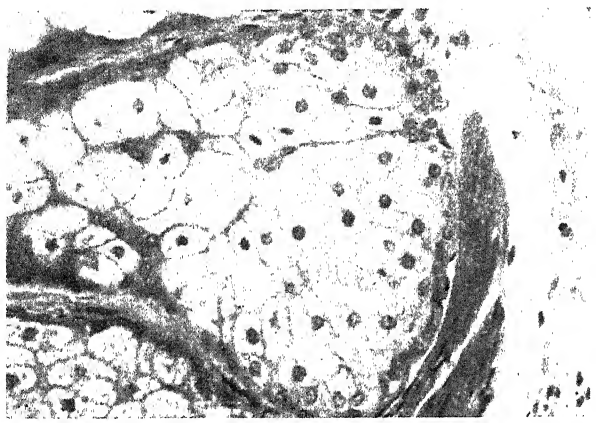
Figures for the rate of growth of individual hairs vary considerably, probably because of the influence of factors mentioned above. A rate of 0.2–0.44 mm per 24 hours in males is usually given, with the higher rate occurring on the scalp. Shaving does not appear to affect the growth rate, nor does hair grow after death.

**Innervation of hair.** Hairs are tactile organs, and are richly innervated as described on page 968. **Blood supply** is via collaterals from the reticular arteriolar plexus to the dermal papilla and from ascending branches to anastomosing networks around the bulb and the inferior segment of the follicle.

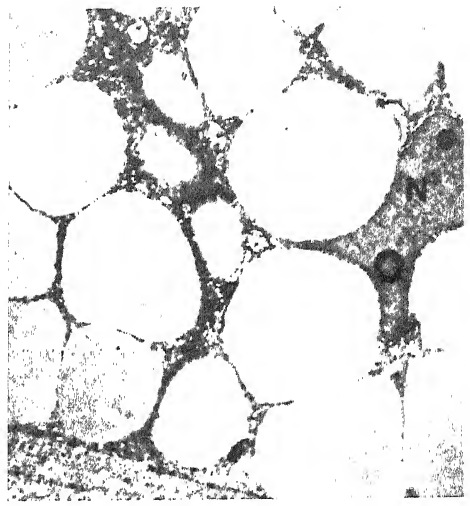
(For further details on the hair follicle see Orfanos et al 1981; Orfanos & Happle 1990.)

## Sebaceous glands

Sebaceous glands are small saccular structures lying (5.1, 46, 56), in the dermis; these, together with the hair follicle and arrector pili muscle, constitute the major part of the pilosebaceous unit. They are present over the whole body except the thick hairless skin of the palm, soles and flexor surfaces of digits. Typically, they consist of a cluster of secretory acini opening by a short common duct into the dermal pilary canal of the hair follicle (5.1, 45) into which they liberate their secretory product, *sebum*. In some areas of thin skin lacking hair follicles, their ducts open instead directly onto the skin surface, for example on the lips and corners of the mouth, the buccal mucosa (Fordyce spots, p. 1688), nipples, female mammary areolae, glans penis, inner surface of the prepuce (glands of Tyson), glans



5.56 A sebaceous gland showing the progression from small polygonal cells around its margins to the large, highly vacuolated cells in the interior of a saccule. The sebum has been extracted by histological processing, leaving an empty frothy appearance in cells about to undergo holocrine secretion. Haematoxylin and eosin. Smooth myocytes of an arrector pili muscle visible on the right. Magnification ×540.



5.57 From central portion of fully differentiated sebaceous gland of an adult. The nucleus of the cell (N) is irregular due to compression by lipid droplets separated only by tenuous cytoplasmic septa. These septa will finally rupture liberating the cellular contents into the duct. Magnification ×3528. (Reproduced from Breathnach 1971, with permission.)

clitoridis and labia minora. At the margins of the eyelids, the large complex palpebral tarsal glands (Meibomian glands) are of this type, and also occurring in the eyelid is the *unicellular sebaceous gland of Wolff* (Pelfini et al 1969) which is actually a melanocyte full of lipid droplets. They are also present in the external auditory meatus.

In general, numbers of sebaceous glands in any given area reflect the distribution of hair follicles, ranging from an average of about 100/cm$^2$ over most of the body to as many as 400–900/cm$^2$ on the face and scalp. They are also numerous in the midline of the back. Individual sebaceous glands are particularly large on the face, around the external auditory meatus, chest and shoulders, and on the anogenital surfaces. Those of much of the face are related to very small vellus hairs whose investing follicles have particularly wide apertures.

**Microstructure.** Microscopically, the glandular acini are seen to be invested by a basal lamina supported by a thin dermal capsule and a rich capillary network. Within this, each acinus is lined by a single layer of small, flat, polygonal epithelial cells which ultrastructurally resemble undifferentiated basal keratinocytes of interfollicular epidermis. They possess euchromatic nuclei and large nucleoli, scattered keratin filaments, free ribosomes, agranular endoplasmic reticulum and rounded mitochondria, and are attached to each other by desmosomes. Functionally, they are mitotically active stem cells whose offspring move gradually towards the centre of the acinus increasing in volume, and accumulating increasingly swollen lipidic vacuoles (**5.57**), which some observers describe as membrane-limited, and others as non-limited. The nuclei become pyknotic as the cells mature, and finally the huge distended cells disintegrate, filling the central cavity and its effluent duct with a mass of fatty cellular debris. This mode of secretion, involving the total destruction of the glandular cells, is described as *holocrine* (p. 74) and takes about 2 to 3 weeks. The secretory products pass through a wide duct lined with keratinized squamous epithelium into the infundibulum of the hair follicle and thence on to the surface of the hair and the general epidermis.

The normal functions of sebum are a matter for discussion. It forms a major component of the *skin surface lipids*, the remainder being provided by the interfollicular epidermis. The lipids provide a protective coating on hairs, possibly assist waterproofing of the epidermis, discourage blood-sucking ectoparasites and contribute to characteristic body odour, a feature which in our ancestral conditions may have possessed strong positive social connotations and in the newborn could play a part in the relationship between mother and child. A general antibacterial activity has been postulated, but the presence in sebum of triglycerides which can be hydrolysed by bacteria, including *Propionobacterium acnes*, argues against this.

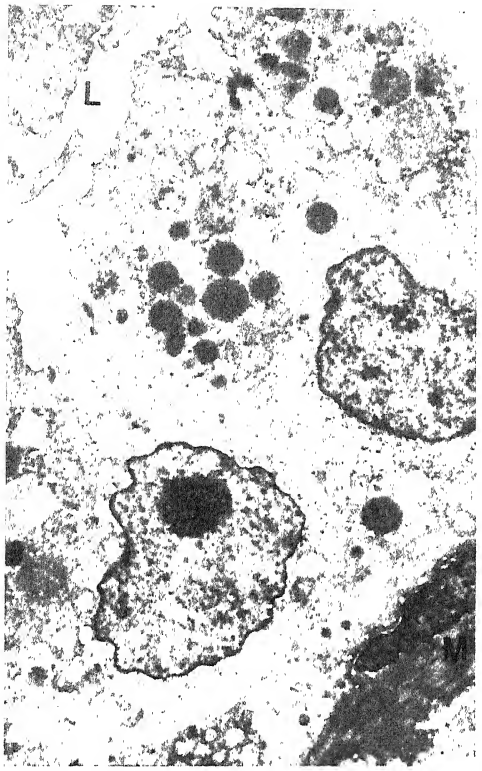
**Sebum and sebaceous activity.** When first formed, sebum is a complex mixture of which over 50% is di- and triglycerides, with smaller proportions of wax esters, squalene, cholesterol esters, cholesterol and free fatty acids, mainly of 16-carbon atom chain length; phospholipids are not present (Stewart et al 1983; Stewart 1992). At birth, sebaceous glands are quite large, regressing later until stimulated again at puberty. At that time, sebaceous gland growth and secretory activity increase greatly in both males and females, under the influence of androgens (testicular and adrenal), and possibly growth hormone from the adenohypophysis, and thyroid hormone, amongst other factors. Androgens act locally on the gland, and there is no motor innervation. Oestrogens have an effect opposite to that of androgens, and secretion is considerably lower in women, becoming greatly decreased after the age of 50 years.

Little is known of the biosynthesis of sebum because of the holocrine nature of its formation, and its expression from the duct is due to continuous pressure from behind of disintegrating cells, aided possibly by compression due to contraction of the neighbouring arrector pili muscles. Excessive amounts of sebum may become impacted within the duct, and this, associated with hyperkeratinization, may lead to it being blocked to form a 'comedone', which, becoming infected and inflamed, is the primary lesion of acne. There are now sufficient therapeutic agents available, both topical and systemic, which are largely capable of ameliorating and curing acne. What is needed in addition is an awareness of this amongst practitioners and patients, the effort to apply them and a general change in attitude towards this most distressing of skin diseases.

## Apocrine glands

The apocrine glands are particularly large glands of the dermis or hypodermis, classed as a type of sweat gland, but, since they develop as outgrowths of the hair follicle and discharge secretion into the hair canal, they are appropriately considered here. They are widely distributed over the skin of mammals generally, including lower primates, in whom they serve a thermoregulatory function, but in the adult human are present in only a few areas, namely the axillae, perianal region, areolae, periumbilical skin, prepuce, scrotum, mons pubis and labia minora. Ceruminous glands of the external auditory meatus and the ciliary glands of the palpebral margins (glands of Moll) are also usually included in this category. However, their secretions are quite different and these glands should be considered as distinct, specialized subtypes (for details see p. 1369 and **8.459**, respectively).

The gland consists of a basal secretory coil and a straight duct which opens into the pilary canal above the duct of the sebaceous gland, or directly on to the skin surface if there is no associated hair.



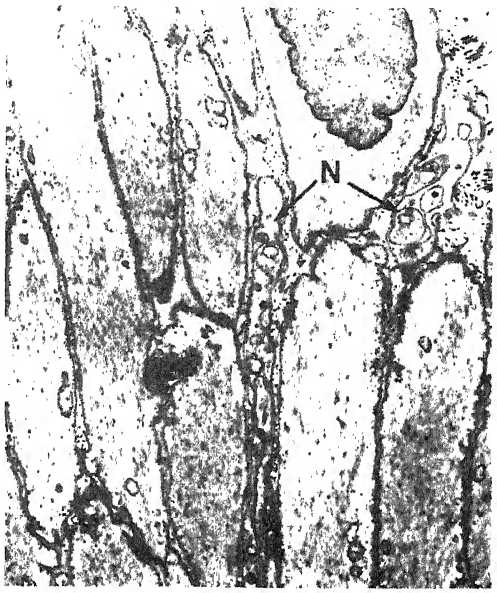

5.58 Secretory coil of axillary apocrine gland. Secretory cells containing granules project into the lumen (L), and rest upon myoepithelial cells (M). Magnification × 6000.

The secretory region may be as much as 2 mm wide and its coils often anastomose with each other to form a labyrinthine network. Each coil is lined by cuboidal secretory cells with apical caps of cytoplasm projecting into the lumen beyond terminal junctional complexes, and resting upon a layer of myoepitheliocytes (5.58). The whole complex of cells is limited by a thick basal lamina, and outside this is a connective tissue capsule, rich in capillaries and containing nerve terminals. The secretory cells contain vacuoles, vesicles and dense granules of varying size and internal structure, whose numbers and character vary with the cycle of synthesis and discharge. 'Clear cells' with few organelles are sporadically present basally among the myoepitheliocytes. The mechanism of secretion is still not entirely clear but may involve a number of different processes (see Hashimoto 1978), including merocrine secretion of granules, detachment or pinching off of apical caps or complete holocrine disintegration of the cells. Secretion is pulsatile, the thick, milky proteinaceous product being projected into and along the duct by contraction of the myoepitheliocytes.

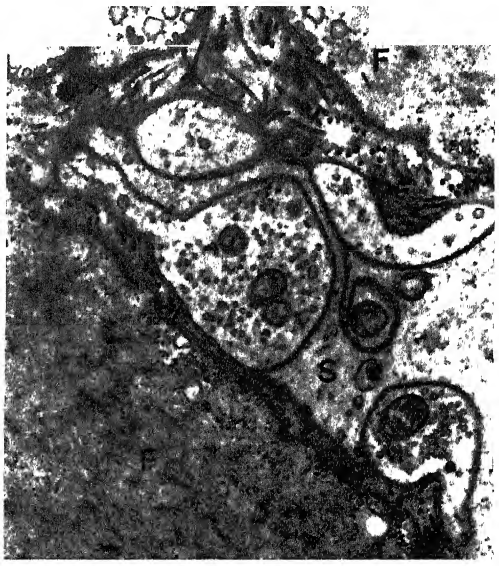
Apocrine activity is minimal before puberty, after which it is androgen dependent and responsive to emotional stimuli. It is controlled by adrenergic nerves, and is sensitive to epinephrine and norepinephrine. The secretion as it emerges is sterile and odourless, but it undergoes bacterial decomposition to generate potent odorous compounds, musky or urinous in smell, including short-chain fatty acids, steroids such as 5 $\alpha$-androstenone, etc. In many animals these are potent pheromonal signals important in courtship, parental and territorial behaviour, as well as in various other aspects of social life. Their role for modern humans is less certain, although there has been much speculation on the potency of, for example, axillary odours on the more subtle aspects of human interactions (see Gower et al 1987).

## Arrector pili muscles

The arrector pili muscles are small fasciculi of smooth muscle cells which form diagonal links between the dermal sheaths of hair follicles and the papillary layer of the dermis (5.1, 45, 56). They are attached




5.59A. Arrector pili muscle. Fibres are sectioned tangentially, and nerve terminals (N) and collagen fibrils are disposed among them. Magnification × 6000.




5.59B. Arrector pili muscle. Between the fibres (F) are axonal terminals enclosed in Schwann cell cytoplasm (S). The terminals contain clear-cored vesicles and mitochondria. Magnification × 15 600.

to the bulge region of the follicles by means of elastin fibrils, and are directed obliquely and superficially towards the side to which the hair slopes. The sebaceous gland occupies the angle between the muscle and the hair follicle. Contraction, therefore, tends to pull the hair into a more vertical position and to elevate the epidermis surrounding it into a small hillock, while dimpling the surface where the muscle is inserted superficially, giving the appearance of 'goose-flesh'. Arrector pili muscles are absent from facial, axillary, and pubic hairs and from eyelashes and eyebrows, and the hairs around nostrils and the external auditory meati.

The closely-packed myocytes are separated by narrow intervals containing collagen fibres and non-myelinated Schwann cell–axonal complexes (5.59A, B). They exhibit the typical features of smooth-

muscle cells—loose bundles of myofilaments associated with dense foci orientated along the long axis of the cell, with glycogen particles and pinocytotic vesicles distributed just within the plasma membrane, which has a basal lamina. The innervating axonal terminals contain mainly clear-cored vesicles (**5.59B**), and are noradrenergic sympathetic.

These muscles by virtue of their position could help to express the secretions of sebaceous glands, though it is doubtful if they act in this way. In many mammals, piloerection is a means of signalling aggression, fear, and other social responses, and can have a thermoregulatory function by trapping an insulating layer of air within the fur.

## NAIL UNIT

The nail unit has five components:

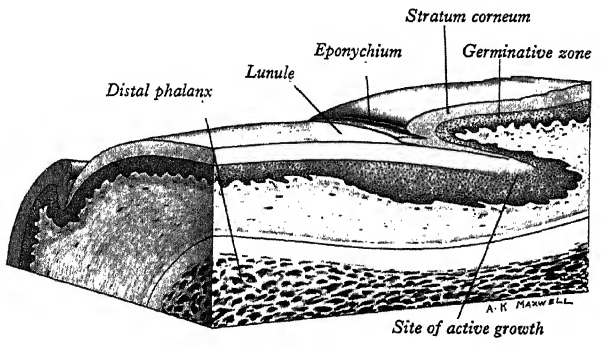
- the *nail plate*, a horny translucent plate on the extensor surface of the distal segment of each digit
- the *matrix*, the proximal extension of the nail plate underneath
- a *proximal nail fold*
- a *nail bed* on which the nail plate rests
- the *hyponychium*, which underlies the free distal edge of the nail plate, and which is separated from the adjacent volar skin of the digital tip by a shallow *distal nail groove*.

The sides of the nail plate are bordered by *lateral nail folds* continuous with the proximal fold, and *lateral* and *proximal nail grooves* marking the conjunction (**5.60**). A fold of skin, the *eponychium*, borders the proximal edge of the exposed nail.

### Nail plate

The nail plate is approximately rectangular in shape (**5.60**), and is mostly convex in both longitudinal and transverse axes, though there is much variation, between both individuals and the different digits of one person. The thickness increases proximodistally from about 0.7 mm to 1.6 mm, and the terminal thickness can vary considerably from individual to individual (Johnson et al 1991). The surface of the nail plate may show fine longitudinal ridges, and its under surface is grooved by corresponding ridges of the nail bed. Disturbances of growth pattern or disease may lead to transverse ridging or grooves (Beau's lines; Meuhrcke lines), and minute trapped air-bubbles may produce white flecks. These defects move distally with growth of the plate. The colour of the nail plate is generally translucent pink, except proximally, always on the pollex, and to a varying extent on the other digits, depending upon manicuring, where a crescentic white opaque area, the *lunule*, is present, emerging from under the proximal nail fold. Its alleged greater surface area on the dominant thumb is said to be a mark of handedness.

Nails are homologous with the stratum corneum of the general epidermis, consisting of compacted, anucleate, keratin-filled squames which are variably described as being disposed in two or three horizontal layers, depending upon views as to their origins from particular parts of the nail unit (see below). Ultrastructurally, the squames contain closely-packed filaments which lie transversely to the direction of proximodistal growth, and are embedded in a dense protein matrix, which also forms a dense marginal band within the tortuous and interlocking cell membranes. Hashimoto (1971) described the squames as being connected by desmosomes and gap junctions, but others (Parent et al 1985) observed only desmosomes. All authors, quoting Hashimoto (1971a), state that keratohyalin is not involved in keratinization of postnatal nail, which is interesting in view of the fact that it is most certainly involved in this process in fetal nail (Hashimoto et al 1966; Breathnach 1971). This point could bear re-examination. Squames are not shed from the nail plate surface, unlike the general epidermis.



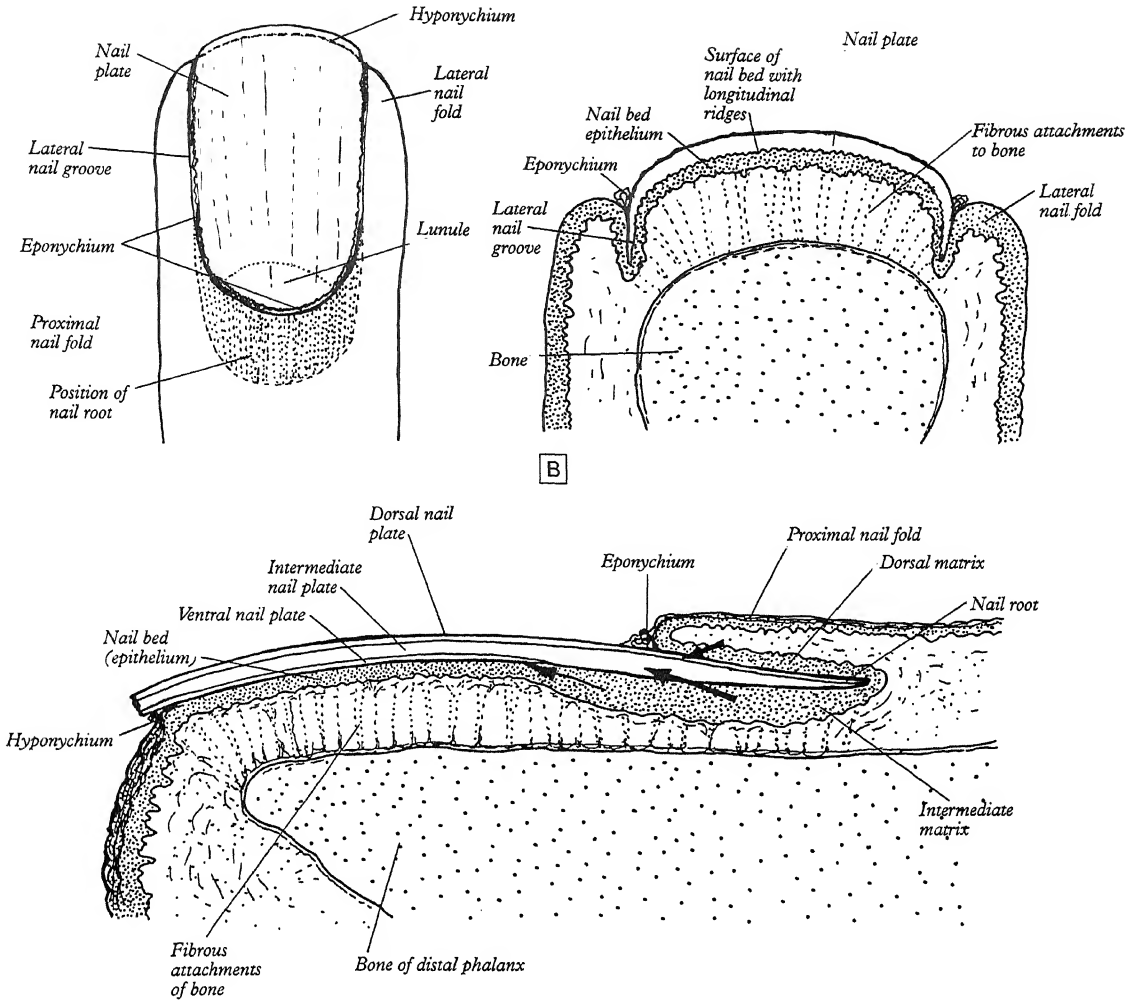

408 **5.60** Longitudinal section through the root of a nail.

The nail plate has a high content of sulphur-containing matrix proteins, but a lipid content less than that of general stratum corneum. A variety of mineral elements is present in nail, among which is calcium, though this is not responsible for the hardness of nail. This is related rather to the arrangement of the layers of squames, their mutual adhesion, and the disposition of their internal fibres (Zaias 1990). Analysis of metal elements in nail has forensic importance in diagnosing excessive ingestion, for example of arsenic, criminally administered. The water content of nail is low, but nail is 10 times as permeable to water as general epidermis (Baden 1970). Elasticity of the nail plate is related to its degree of hydration.

**Proximal nail fold.** Proximally, the nail plate extends under the *proximal nail fold*, forming an angle with it (Lovibond's angle), which is less than 180°. The fold is composed of two epidermal layers, superficial and deep, with a core of dermis in between. The epidermis of the superficial layer lacks hair follicles and epidermal ridges, and its stratum corneum extends over the nail plate for a little distance as the *cuticle* or *eponychium*. The ventral layer merges deeply with the *matrix* which produces the greater part of the nail plate.

**Matrix.** On section, the matrix is seen as a wedge of cells with its apex proximal, in which the deeper part of the nail plate is embedded (**5.61, 62**). Those cells lying dorsal to the plate are referred to as the *dorsal matrix*, and are continuous with the ventral epithelium of the proximal nail fold. Those lying ventral are known as the *ventral matrix*, which is continuous distally with the nail bed. From the apical region of the matrix fine bundles of anchoring filaments extend into the dermis. The matrix epithelium consists of typical basal and spinous layer keratinocytes with axes directed diagonally distally, among which melanocytes and Langerhans cells are intermingled. The keratinocytes produce membrane coating granules, but not keratohyalin granules (Hashimoto, 1971), although granules like those of keratinizing oral epithelia have been reported. Keratinized cells of dorsal and ventral matrices are steadily extruded distally to form the nail plate with the major contribution coming from the ventral matrix. This continues into the **nail bed** at the distal edge of the lunule, which is formed by the distal portion of the ventral matrix overlain by the nail plate.

**Nail bed.** This underlies the nail plate from the lunule to the *hyponychium*, and its surface is ridged and grooved longitudinally in correspondence with a similar pattern on the under surface of the nail plate, which results in a tight interlocking coupling of the two that prevents the invasion of microbes and the impaction of debris underneath the nail. The pattern is imposed by underlying dermal ridges, and is thought to represent the finger print pattern elsewhere. The epidermis of the nail bed consists of two to three layers of nucleated cells lacking keratohyalin granules, and a thin keratinized layer which moves distally with the growing nail plate. Until recently, the nail plate was thought to be derived entirely from the matrix, growing distally over the nail bed, and carrying the cornified cells of the latter along with it to be shed distally. In this scheme, the nail bed was regarded as providing a gliding surface for an already fully formed growing nail plate. Now, however, it is generally thought that nail bed cells differentiate towards the nail plate, contributing a significant component to it ventrally. The plate, therefore, is now thought to consist of three horizontal layers—a dorsal one from the dorsal matrix, an intermediate one from the ventral matrix and a ventral one from the nail bed. Authorities, however, are not in agreement as to the extent of contribution of the three components (Forslind & Thyresson, 1975; Parent et al 1985; Ziais 1990; Baran et al 1991; Johnson et al 1991). Beneath the epithelium of the nail bed is a dermis anchored to the periosteum of the distal phalanx without any intervening subcutis. It forms a distinct compartment and because of this, infections of the nail bed, or other local sources of rise of pressure (e.g. haematoma), may cause severe pain only relieved by excision of part or all of the nail plate. The dermis is




5.61 A–C. Diagram showing the organization and terminology of the structures associated with a fingernail. (A) shows the appearance of the dorsal side, indicating the extent of the hidden nail root. (B) depicts a cross section of a fingertip, and (C) a longitudinal section through a nail and its surrounding structures, indicating the areas of formation of the nail plate from the different areas of matrix.

richly vascularized, including large arteriovenous shunts (glomera), and numerous sensory nerve endings, including Merkel terminals and Meissner corpuscles. Fingernails subserve an important tactile function, providing support and counterpressure for the digital pad, thereby aiding manipulation. Spatulate (flat) nails are found in all primate species, and are an evolutionary development from sharp claws. They represent a thin superficial protective stratum of the tetrapod claw.

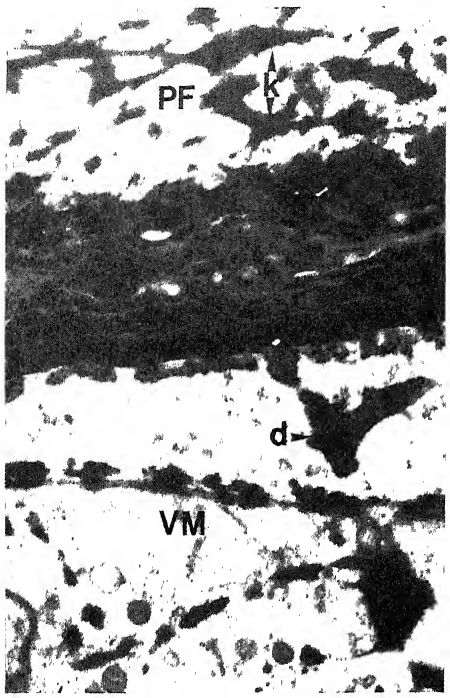
**Hyponychium.** An area of epidermis which underlies the edge of the nail plate, it extends from the nail bed to the distal groove which marks its continuation into the general epidermis of the finger tip. The basal layer cells have a semi-palisade arrangement, the granulosa cells are packed with keratohyalin granules and the stratum corneum is undulant and continually being shed. The hyponychium provides an important defence against entry of bacteria underneath the free edge of the nail plate, and may be damaged by too vigorous cleaning in this situation.

**Growth of nail.** The growth of nail is determined by the turnover rate of the matrix cells, which varies with digit, age, environmental temperature and season, time of day, nutritional status, trauma, such as biting, and various diseases. Generally, its speed is related to the length of the digit, being fastest (about 0.1 mm per day) in the middle finger of the hand, and slowest in the little finger. Fingernails grow up to four times faster than toenails, quicker in summer than in winter, and faster in the young than in the old. Left unclipped and protected from erosion, nails can grow to considerable lengths, and in previous Chinese cultures they were allowed to do so by the wealthy classes as an indication that they were waited upon for everything. The long nails were protected by elaborately jewelled and decorated sheaths. Painting the toenails red was said to be a signal of menstrual status in the seraglio.

## ECCRINE SWEAT GLANDS (5.63–67)

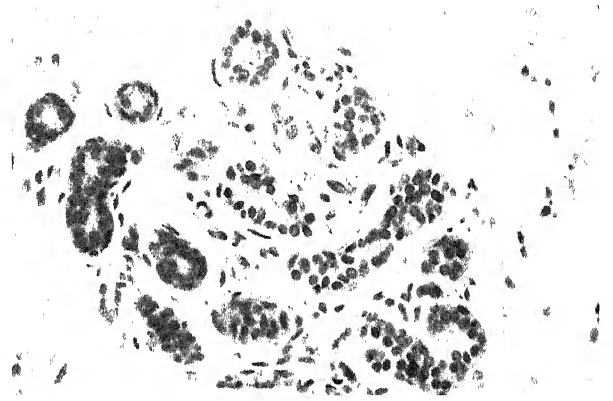
The eccrine sweat glands are long unbranched tubular structures, each with a highly coiled, wider secretory portion (the body or fundus, up to 0.4 μm in diameter; 5.63) situated deep in the dermis or hypodermis and a narrower, straight or slightly helical ductular portion, which in the deeper layers of the dermis is convoluted or twisted (5.1, 45). The walls of the duct fuse with the base of epidermal (rete) papillae and the lumen passes between the keratinocytes often, particularly in thick hairless skin, in a tight spiral (5.3), to open via a rounded aperture onto the skin surface (5.19). In thick hairless skin, they discharge by a regular series of punctae along the centre lines of friction ridges, incidentally providing markers of fingerprint patterns for forensic purposes. Eccrine glands have an important thermoregulatory function, and their secretion enhances grip and sensitivity of the palms and soles.

Eccrine glands are absent from the tympanic membrane, margins of the lips, nail bed, nipple, inner preputial surface, labia minora,




5.62 Longitudinal section of nail organ of a fetus aged 16 weeks. Dorsal to the keratinized squames of the nail plate is a cell of the proximal nail fold (PF) with typical keratohyalin granules (k) and ventral to them a cell of the ventral matrix (VM) with dense cytoplasmic granules (D) similar to those seen in areas of keratinizing oral epithelium. Magnification ×10 080. (Reproduced from Breathnach 1971, with permission.)

glans penis and glans clitoridis. Elsewhere they are numerous, their frequency ranging from 80 to over 600/cm$^2$, depending on position and genetic variation; the total number lies between 1.6 and 4.5 million (Millington & Wilkinson 1983; Ito 1988). Numbers are greatest on the plantar skin of the feet, but there are also many on the face and flexor aspects of the hands, while the surfaces of the limbs generally have the fewest. Racial groups indigenous to warmer climates tend to have more than those of cooler geographical areas. Ito (1988) has described apo-eccrine glands with features of both apocrine and eccrine glands in the human axilla.



5.63 Section through the basal coil of a sweat gland showing the wider secretory portion and the narrower initial region of the sweat duct composed of two layers of small cuboidal cells. Haematoxylin and eosin. Magnification ×540.

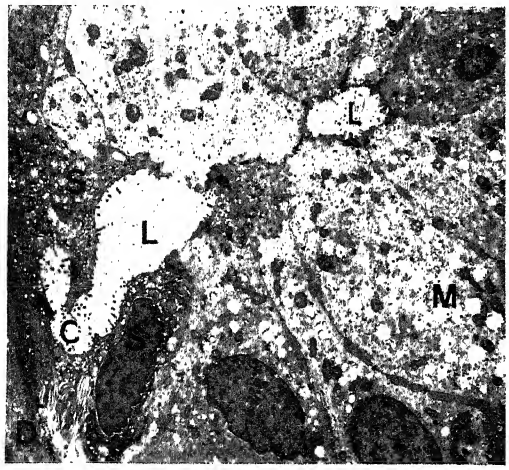
## Cell types

Microscopically the secretory coil consists of a pseudostratified epithelium enclosing a lumen with intercellular canaliculi resting on a basal lamina and enclosed by a thin fibrous dermal sheath. There are three types of cell:

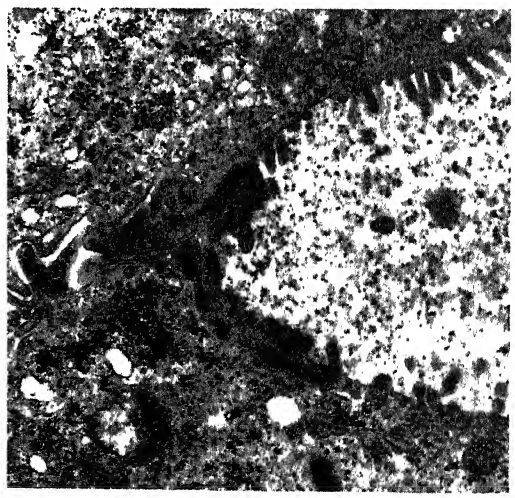
- *clear* (serous) cells from which most of the secretion derives
- *dark* (mucoid) cells
- *myoepitheliocytes.*

**Clear cells.** These are approximately pyramidal in shape, with their bases resting on the basal lamina or myoepitheliocytes, and their microvillus-covered apical plasma membranes lining the intercellular canaliculi. The lateral plasma membrane is highly folded, interdigitating with folds of apposed clear cells (5.66), as is also the basal plasma membrane where it abuts on the basal lamina. The cytoplasm is rich in glycogen granules and mitochondria, and granular endoplasmic reticulum and a small Golgi complex are present, but few other formed organelles. The nucleus is rounded and moderately euchromatic.

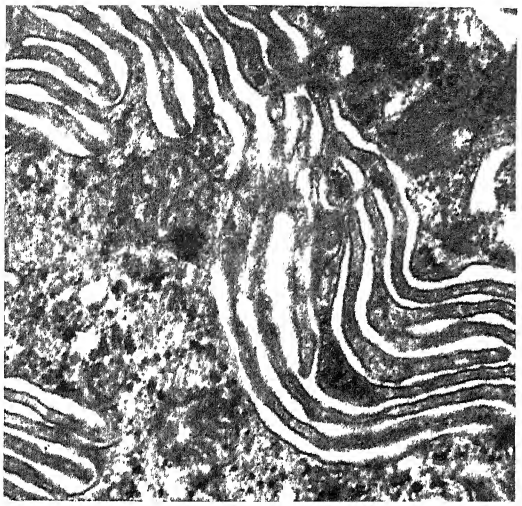
**Dark cells.** These are also pyramidal, with their broad ends facing and forming the greater extent of the lining of the main lumen. The cytoplasm contains a well-developed Golgi complex, numerous



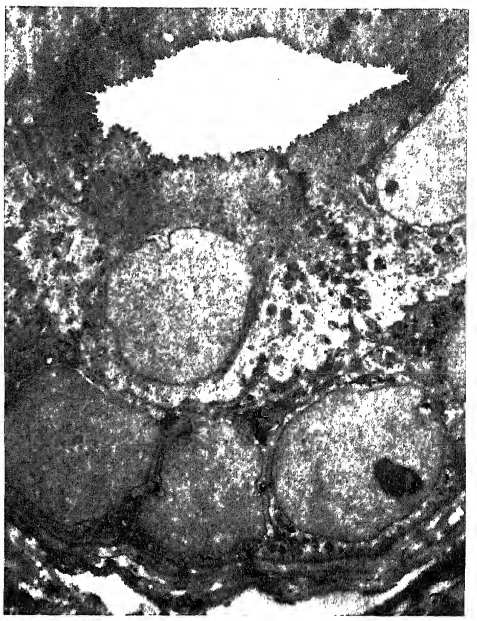

5.64 Portion of secretory coil of eccrine gland. Serous (S) and mucous (M) cells surround the lumen (L) from which an intercellular canaliculus (C) extends between serous cells. D, dermis. Magnification ×2656. (Reproduced from Breathnach 1971, with permission.)



5.65 Eccrine sweat gland. Portions of two serous cells are seen bounding the lumen. Note microvilli on the luminal plasma membrane. Magnification ×14 000. (Reproduced from Breathnach 1971, with permission.)



5.66 Eccrine sweat gland. This illustrates interdigitation of long villous processes of plasma membranes of apposed serous cells. Magnification ×33 440. (Reproduced from Breathnach 1971, with permission.)



5.67 Transverse section of upper portion of intradermal sweat duct, which is composed of two cellular layers, basal and luminal. The supranuclear portions of the cells bounding the lumen appear 'clear' because the cytoplasm here contains mainly filaments, and this zone corresponds to the cuticular border of light microscopy. Magnification × 4480. (Reproduced from Breathnach 1971, with permission.)

vacuoles and vesicles and dense granules of different sizes, indicating a type of secretion different to that of the clear cells.

**Myoepitheliocytes.** Similar to those elsewhere (p. 780), these form an incomplete layer along the basal lamina with processes of clear cells in between. The infranuclear region of the cytoplasm is almost entirely occupied by myofilaments, the bulk of the other organelles lying superior and lateral to the nucleus.

**Sweat ducts.** The *intradermal sweat duct* is formed of two cell layers, an outer basal layer and an inner layer of luminal cells connected by numerous desmosomes, and with microvilli along the luminal border. The supranuclear cytoplasm is highly filamentous, corresponding to the eosinophilic 'cuticular border' of light microscopy. The *intraepidermal sweat duct* is twisted, and also consists of essentially two layers of cells which, developmentally, are different to the surrounding general keratinocytes. The outer cells near the surface contain keratohyalin granules and lamellar granules, and undergo typical keratinization. The inner cells from a mid-epidermal level contain numerous vesicles just within the microvillous luminal plasma membrane, undergo an incomplete form of keratinization, and are largely shed into the lumen at the level of the stratum corneum.

## Sweat

Sweat is a clear, odourless fluid, hypotonic to tissue fluid, containing small quantities of many substances, mainly sodium and chloride ions, but also potassium, urea, lactate, amino acids, immunoglobulins and other proteins, epidermal growth factor, bicarbonate, calcium ions, etc. Heavy metals and various organic compounds are eliminated in sweat, the greater part of which is thought to be produced by the clear cells, the function of the dark cells being uncertain. The function of the myoepitheliocytes is equally obscure, but it has been suggested they provide support for the secretory cells against overdistension when large amounts of fluid are being secreted, squeeze fluid into the canaliculi and lumen, or actually separate when contracted thus exposing a greater area of the clear cell to the basal lamina and dermal extracellular fluid. When initially secreted, the fluid is similar in composition to tissue fluid, but it is modified as it passes along the duct by the action mainly of the basal cells which exhibit Na-K-adenosine 5'-triphosphate (ATP)ase activity and resorb sodium and chloride and some water too. The hormone aldosterone enhances this activity. The body's sweat glands are capable of producing up to 10 litres of sweat per day, in response to thermal, emotional, and gustatory stimuli, mediated by non-myelinated sympathetic cholinergic fibres, though the glands also respond to adrenaline (Sato et al 1991). *Thermoregulation* is a complex process involving a heat centre in the hypothalamus reacting to changes in blood temperature and afferent stimuli from the skin, and controlling cutaneous blood supply and the rate and volume of sweat secretion for evaporation at the surface (Clark and Edholm 1985). Excessive sweating can lead to salt depletion.

# IN SKIN

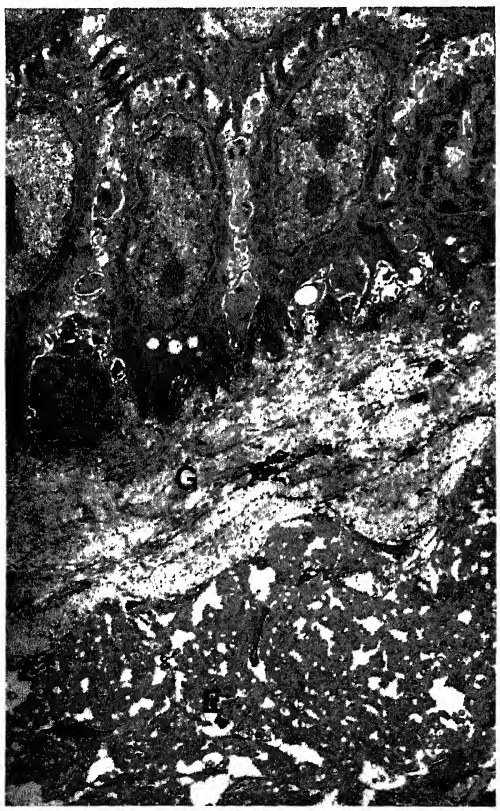
With the increase in the proportion of elderly persons in the population, interest in age-related changes in skin has greatly expanded in recent years (Montagna et al 1979; Balin & Kligman 1989; Greaves & Voorhees 1990). This interest is from both an aesthetic and a pathological point of view. Two main factors, chronological and environmental, are said to be involved in skin ageing, with the former being regarded as more physiological or intrinsic. A major environmental factor is chronic exposure to the sun; referred to as photoageing, and emphasis is laid upon differences between the two since photoageing is to some extent preventable (Gilchrest 1987; Kligman & Lavker 1988). The major features of skin are essentially formed well before birth, and during the first two to three decades of life the main changes are an expansion of its surface area and thickening of the epidermis and dermis, as well as various changes in hair and gland patterns which occur at puberty (see pp. 405, 406). The arrangement and numbers of creases and friction ridges is essentially the same from their early fetal formation onwards, although their sizes increase until cessation of growth. However, from about the third decade onwards there is a gradual change in the appearance and mechanical properties of the skin which reflect natural ageing processes, in old age becoming very marked.

## Intrinsic ageing

Normal human ageing is accompanied by epidermal and dermal atrophy, which result in some changes in the appearance, microstructure and function of the skin. Alterations include wrinkling (see p. 379), dryness, loss of elasticity, thinning and a tendency towards purpura on minor injury. Epidermal atrophy is expressed by general thinning and loss of the basal rete pegs with flattening of the epidermal–dermal junction, resulting in a reduction in contact area between the two which may affect epidermal nutrition. Flattening of

the junction decreases resistance to shear, leading to poor adhesion of epidermis and its separation following minor injury. The thickness of the stratum corneum is not reduced in old age, and its permeability characteristics seem little affected. Epidermal proliferative activity and rate of cell replacement decline with age, the thymidine-labelling index being reduced by up to 50%. Synthesis of vitamin D is reduced with this general decline in activity. After middle age there is a 10–20% decline in the number of melanocytes (Ortonne 1990), and Langerhans cells also become sparser, associated with a reduction in immune responsiveness (Schuler 1991). These alterations in non-keratinocytes may be aggravated by chronic exposure to UV irradiation. Depigmentation and loss of hair with some local increases—eyebrows, nose and ears in males, and face and upper lip in females—are so well known as hardly to require mention. Decrease in function of skin glands associated with degenerative changes has been described.

Dermal changes are mainly responsible for the appearance of aged skin, its stiffness, flaccidity and wrinkling, and loss of extensibility and elasticity (Lapiere 1990). Its general thickness diminishes due to a fall-off in collagen synthesis by a reduced population of fibroblasts, though the relative proportion of Type III collagen increases (Lovell et al 1987). Senile elastosis (**5.68**) is a degenerative condition of collagen which may be partly due to excessive exposure to sun. There is also loss and fragmentation of elastin, and alterations in matrix components, including reduction in glycosaminoglycans. The general cellularity of the dermis decreases with age, and mast cells in particular are reduced in numbers. Vascularization of the skin is also reduced, the capillary loops of the dermal papillae being particularly affected, and the tendency towards small spontaneous purpuric haemorrhages indicates a general fragility of the cutaneous microvasculature. A decrease in sensitivity of sensory perception associated with some loss of specialized receptors occurs. For further reading on this subject, see L'Evêque and Agache (1993).




**5.68** Epidermis and dermis of facial skin of woman aged over 70, to show area of solar elastosis (E), thought to result from damage to collagen due to chronic exposure to solar radiation. It is interesting that a band just beneath the epidermis, known as the Grenz zone (G), is undamaged. Magnification ×3200.

## Photoageing

Reference has already been made to the developing discipline of photobiology (see p. 391), of which photoageing is a major concern because of an association with epidermal cancer, and further details may be found in the literature cited above. The effects of chronic sun exposure on melanocytes (stimulatory) and Langerhans cells (destructive) has received particular attention because of the increasing incidence of malignant melanoma among sun-worshippers, in which reduction in tumour monitoring activity of Langerhans cells may be a factor. (See Gilchrest et al 1979; Thiers et al 1984; Cruz & Bergstresser 1991.)

For descriptive purposes, dermal repair is divisible into three overlapping phases: *inflammation*, *proliferation* and *remodelling* (**5.69**).

## Inflammation

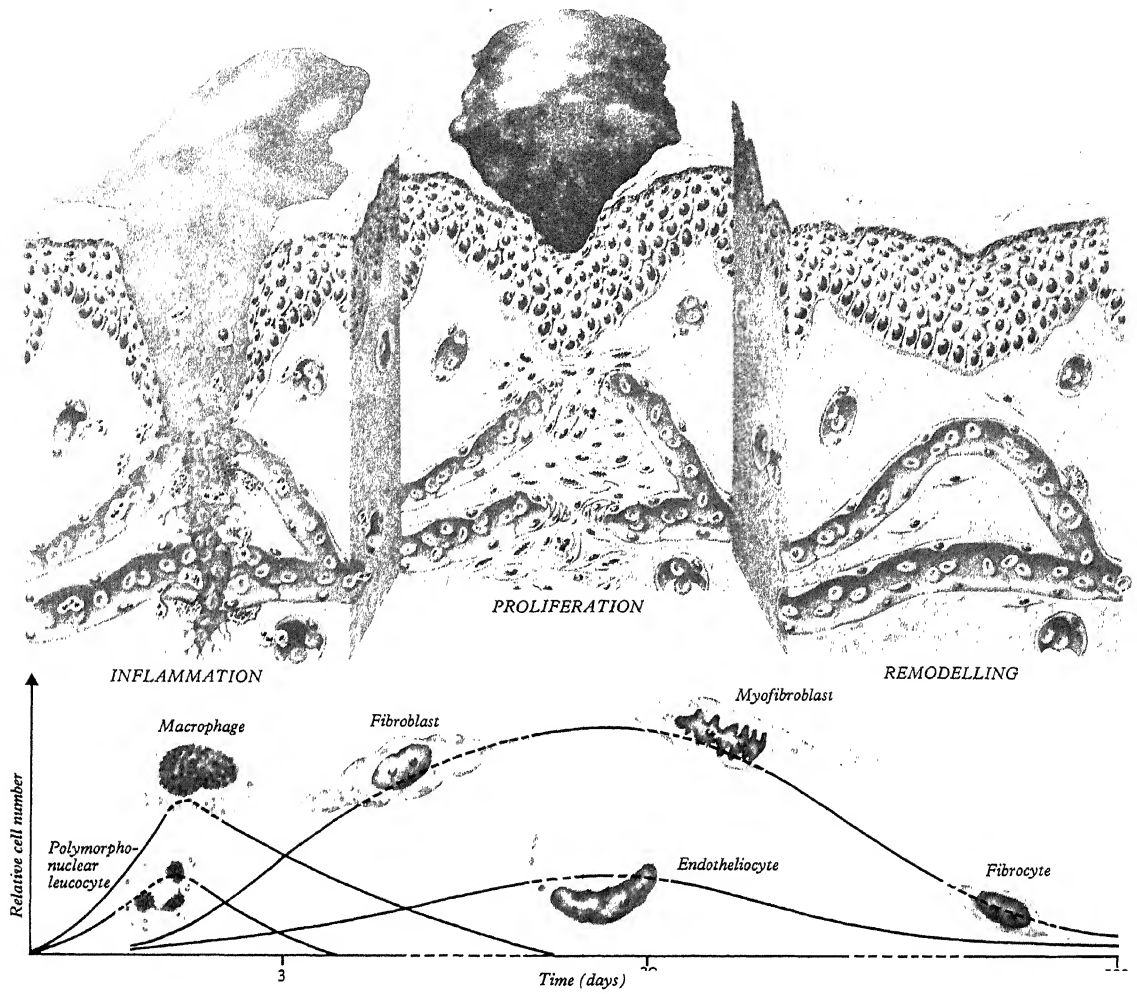
Acute inflammation begins with the activation of platelets and mast cells as an immediate response to injury. During inflammation, haemostasis is achieved, removal of damaged tissue occurs and factors which start the formation of granulation tissue are released or deposited in the wound. Multiple interacting pathways of inflammation (**5.73**) are triggered when dermal blood vessels are injured and end automatically when the inflammatory stimuli dissipate; should these stimuli persist, then inflammation persists and wound healing is delayed.

Tissue injury and bleeding are followed by blood clotting. This involves many complex chemical interactions between components of the extravascular tissue and of blood, including activation of the Hageman factor (for intrinsic coagulation), factor VII (for extrinsic coagulation) and the activation of platelets. These are all responses to the surface adsorption and activation of specific coagulation proenzymes normally inhibited in intact tissues, but free to act in the protease inhibitor-free microenvironment temporarily provided by the wound. Blood clotting is a crucial part of inflammation, because activation of Hageman factor leads to bradykinin generation, initiation of the classic complement cascade (Ghebrehiwet et al 1981) and possibly also to production of anaphylatoxins C3a and C5a (Clark 1985), among many other complex and as yet poorly understood reactions. These anaphylatoxins, together with bradykinin, increase local blood vessel permeability (Williams & Jose 1981), causing leakage of plasma proteins and formation of an extravascular clot. They also stimulate release of the vasoactive mediators histamine and leukotrienes C4 and D4 from mast cells (Hugli & Müller-Eberhard 1978; Stimler et al 1982) and attract neutrophils and monocytes to the wound (Marder et al 1985). As a result of activation the platelets also liberate a host of growth factors which affect the proliferative phase of repair (see below) by stimulating the migration and proliferation of cells involved in this phase and by stimulating the synthesis of extracellular matrix components at the site of the wound. These growth factors include platelet factor-4 (Senior et al 1983), platelet-derived growth factor (PDGF) (Huang et al 1988), transforming growth factor-$\beta$ (TGF-$\beta$) (Assoian 1988), epidermal growth factor (EGF) (Banks 1988), basic fibroblast growth factor (b-FGF) (Fox 1988) and platelet-derived endothelial cell growth factor (Miyazono & Heldin 1989; Pierce et al 1991).

The main function of the neutrophils while at the wound site is the phagocytosis of pathogenic bacteria. Once bacterial contamination has been controlled, neutrophil infiltration ceases and the early inflammatory phase of repair is at an end. In contrast, monocytes, which develop into macrophages on entering the wound bed, remain throughout the entire inflammatory phase. Macrophages are not only phagocytic but also release a host of biologically active materials, including growth factors essential for the initiation and propagation of granulation tissue during the next, proliferative, phase of repair.

## Proliferation

During this stage, cells and intercellular substances increase greatly to form *granulation tissue*. This is a highly vascular material consisting largely of macrophages, pluripotent pericytes, fibroblasts and




5.69 Diagrammatic representation of the normal response of skin to incision showing the changes in relative numbers of different cell types during inflammation, proliferation and remodelling. For further explanation see text.

endothelial cells lining capillaries, all embedded in a matrix of fibronectin, proteoglycans rich in hyaluronic acid, and collagen, which at first is mainly Type III, changing later to Type I. The profuse assemblies of capillaries, which are the main type of blood vessel, give this tissue its 'granular' appearance when incised, hence its name (see **5.70, 71, 72**).

Granulation tissue forms in response to various signals, which may include chemotactic and growth factors, structural molecules and proteases which digest connective tissue matrix (Clark 1985). It forms a nutritive substrate over which the regenerating epidermis can migrate and is gradually replaced by scar tissue. Apart from the absence of osteogenic cells and chondroblasts, granulation tissue resembles the blastema which develops at the site of fracture repair (p. 483).

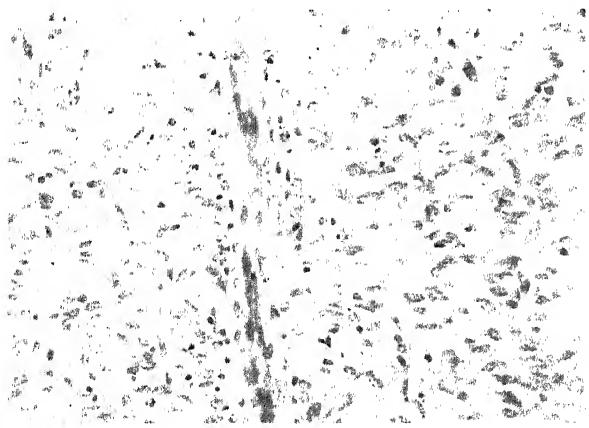
Macrophages, fibroblasts and blood capillaries migrate into the wound bed as a mutually dependent unit termed a *wound module*. In the lead are activated macrophages, followed in sequence by newly differentiated fibroblasts, dividing fibroblasts and capillaries. The macrophages release chemotactic agents which attract pericytes, fibroblasts and endothelial cells into the wound. As the fibroblasts mature they produce a matrix through which other cells can readily migrate and from which delicate new capillaries can obtain mechanical support. As each capillary loop becomes functional it brings nutrients and oxygen to nearby cells, enabling the fibroblasts to secrete materials for the matrix, through which macrophages and other cells can migrate further. The above proliferative and migratory processes are repeated sequentially until the wound bed is filled with granulation tissue. A diagram depicting these complex processes is shown in **5.73**.

There is considerable experimental evidence to support the proposition that macrophages are key cells in dermal repair. They assist in tissue debridement, release chemotactic agents which attract fibroblasts and endothelial cells to the wound site, release growth factors which stimulate these cells to proliferate, and secrete lactate which stimulates collagen synthesis by fibroblasts (Comstock 1970), thus strengthening the tissue which develops within and adjacent to the wound (Silver 1984). Intercellular contacts between macrophages and fibroblasts (**5.74**) suggest that the cells may exert a direct effect on each other. If the migration of macrophages into the wound bed is prevented by anti-inflammatory steroids, or if they are eliminated from it by the application of antimacrophagic serum, the formation of granulation tissue is inhibited (Leibovich & Ross 1975). Inhibition due to anti-inflammatory steroids can be reversed by vitamin A administration, which permits migration of macrophages to the wound site.

During the proliferative phase of repair, fibroblasts of the granulation tissue develop into cells termed *myofibroblasts* (Majno 1979). These cells, which are responsible for wound contraction, the centripetal movement of the wound margin and the consequent reduction of the size of the wound, are immunologically similar to smooth muscle cells, contain peripherally located microfilaments and become linked together by desmosomes and other intercellular contacts (**5.75**). Links between the cells and their substrates have also been found (Ryan et al 1974). Intracytoplasmic filaments of

5.70 Low magnification light micrograph of part of the site of a full-thickness excised lesion produced in porcine skin, 3 days after injury, during the inflammatory phase of repair. Intact skin can be seen on the right. Polymorphonuclear leucocytes and macrophages are present beneath the exudate covering the wound bed, and epidermal migration from the intact skin has already commenced. Stained with haematoxylin and eosin. Magnification x 135. (Material supplied by S Young and photographed by Kevin Fitzpatrick, Department of Anatomy, UMDS, Guy's Campus, London.)

5.71 Low magnification light micrograph of part of the site of a full-thickness excised lesion produced in porcine skin, 10 days after injury, during the proliferative phase of repair. Granulation tissue, over which epidermal cells have migrated, fills the wound bed. Stained with haematoxylin and eosin. Magnification x 135. (Material supplied by S Young and photographed by Kevin Fitzpatrick, Department of Anatomy, UMDS, Guy's Campus, London.)

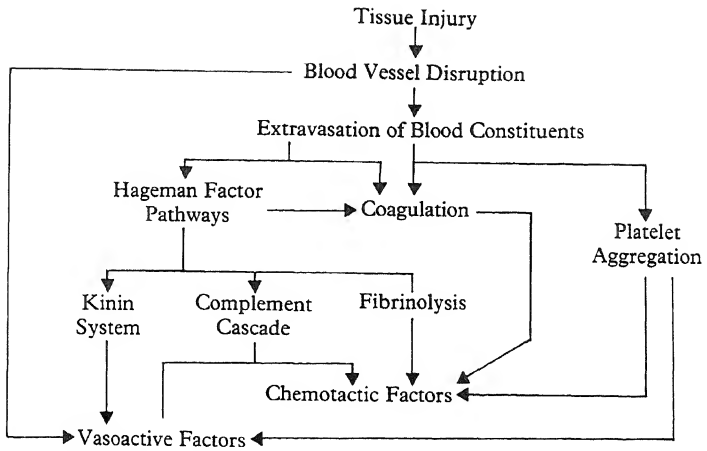


5.72 High magnification light micrograph of granulation tissue, 10 days after the production of a full-thickness excised lesion in porcine skin. The tissue is well vascularized and contains many myofibroblasts, orientated approximately parallel to the base of the wound and at right angles to the majority of the blood vessels. Magnification x 300. (Material supplied by S Young and photographed by Kevin Fitzpatrick, Department of Anatomy, UMDS, Guy's Campus, London.)

actin and vinculin form co-linear assemblies, each termed a *fibronexus* (5.75), with extracellular matrix fibrils of fibronectin (Singer 1979) and Types I and III procollagen (Furcht et al 1980). It has been suggested (Singer et al 1984) that a fibronexus is a cohesive complex which transmits the collective forces generated by contraction of all the myofibroblasts of the granulation tissue to the wound margins, thereby effecting wound contraction.

The mechanism by which myofibroblasts or fibroblasts generate the contractile force needed for wound contraction is still unresolved. According to Gabbiani et al (1971) the contractile force is due to the muscle-like cellular contraction of myoblasts (the cell contraction-myoblast theory) whereas more recently the cell traction-fibroblast theory has been proposed (Ehrlich & Rajaratnam 1990). According to the latter, wound contraction is due to the traction-like activity of fibroblasts on the matrix of the wound bed.

**Angiogenesis.** This is a vital part of the proliferative phrase of dermal repair. Without it invasion of the wound bed by macrophages and fibroblasts would cease through lack of oxygen and nutrients. In vitro studies have shown that capillary endothelial cells release collagenase in response to angiogenic factors. This degrades the collagen of the basement membrane which later fragments, permitting migration of endothelial cells into the perivascular spaces, where they form buds which are added to by the proliferation of cells within and near the parent vessel (Kalebic et al 1983). During dermal repair these buds grow rapidly towards the free surface,




5.73 Mediator pathways of inflammation initiated by tissue injury. (After Clark 1985.)

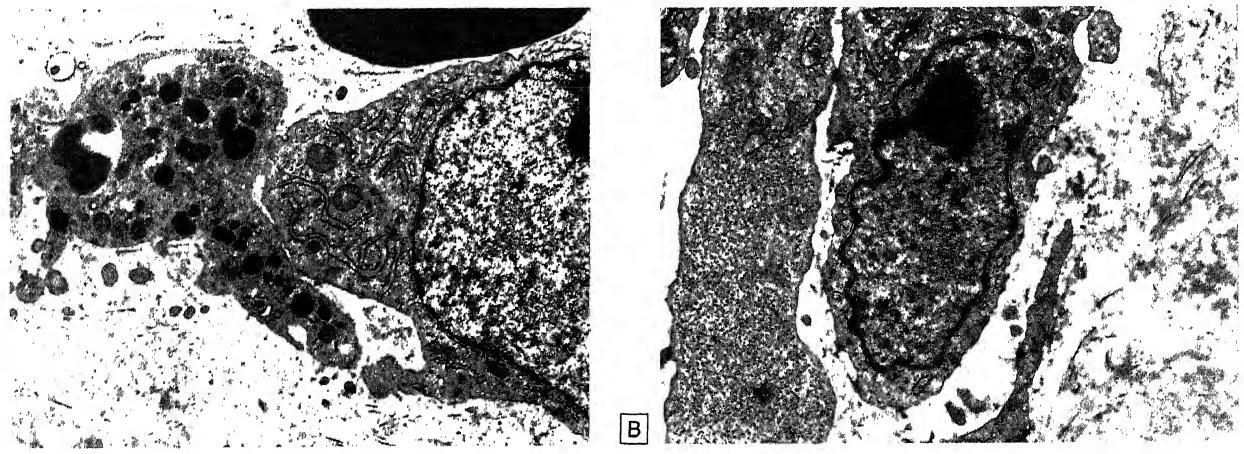
where they branch at their tips and unite to form functional capillary loops. New buds then develop on these loops so that a superficial capillary plexus rapidly forms in the granulation tissue (5.76).

Although the factors responsible for angiogenesis during dermal repair remain unidentified, several candidates have been proposed. These include a macrophage-derived growth factor known to stimulate proliferation of endothelial cells in vitro (Martin et al 1981), low oxygen tension (Remensnyder & Majno 1968), lactic acid (Imre 1964), biogenic amines (Zauberman et al 1969) and hepatocyte growth factor. The last of these, also known as scatter factor, is a powerful mitogen and motility factor which acts through the tyrosine kinase receptor encoded by the metabolic equivalent (MET) proto-oncogene, stimulating endothelial cells to proliferate and migrate (Bussolino et al 1992). Endothelial migration may be more significant than proliferation during angiogenesis after injury. If so, then chemotactic factors will play a key role in vivo. Such factors include platelet-derived substances (Wall et al 1978), heparin (Azizkhan et al 1980) and fibronectin (Bowersox & Sorgente 1982). Successful angiogenesis depends not only on chemotactic and mitogenic factors, but also on the presence of a suitable substrate over which migration of endothelial cells can occur. This may be produced, at least partly, by the endothelial cells themselves, since they have been shown to synthesize fibronectin (Birdwell et al 1978) and collagen (Madri & Stenn 1982).

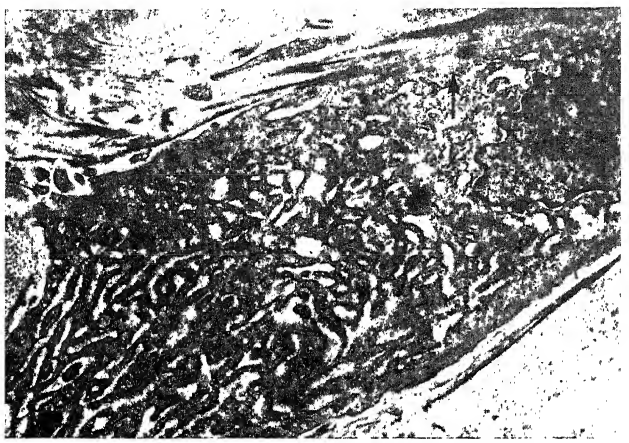
## Remodelling

Just as the proliferative overlaps the inflammatory phase, so remodelling overlaps proliferation. During remodelling the highly cellular and highly vascular granulation tissue is gradually replaced by scar tissue with few cells and blood vessels. During the process of remodelling, which may occupy months or even years, most of the fibronectin is removed from the matrix and there is a slow accumulation of large bundles of Type I collagen fibres which, as they form cross-links, increase the tensile strength of the scar tissue. Changes in the arrangement of the collagen with time after injury have been studied by scanning electron microscopy (Forrester 1973). When it first appears in the granulation tissue of the wound bed it forms randomly arranged fibrils, which gradually develop into large irregular masses without evidence of any fibrillar substructure. The absence of the characteristic pattern found in uninjured dermis may be associated with the decrease in extensibility and tensile strength which are typical of scar tissue. During subsequent remodelling, the orientation of fibres becomes less random and its strength increases. This change may be caused by the action of mechanical forces exerted on the scar during normal usage to produce orientation of the collagen fibrils in the scar tissue and improve its mechanical function, so that it resembles uninjured dermis more closely.

Such forces may also produce a piezo-electric effect which affects the arrangement of collagen fibrils and fibres. There is now convincing evidence that the pattern of collagen within granulation and scar tissue can be altered by local forces, i.e. that remodelling occurs (Forrester 1973). As long ago as 1892 Wolff noted that bone responded structurally to functional demands. It is likely that scar tissue responds in a similar manner.




5.74 Electron micrograph showing intercellular contact between: (A) a macrophage and fibroblast in a healing, full thickness skin lesion, 3 days after trauma; (B) two myofibroblasts in a similar lesion, 7 days after trauma. Magnification ×8000. (Provided by Rachel Hickman, Department of Anatomy, UMDS, Guy's Campus, London.)



5.75 Electron micrograph showing a fibronexus, a region at the surface of a myofibroblast where there is an alignment between intracellular filaments and fibrils of the extracellular matrix (arrow). Magnification × 8000. (Provided by Rachel Hickman, Department of Anatomy, UMDS, Guy's Campus, London.)

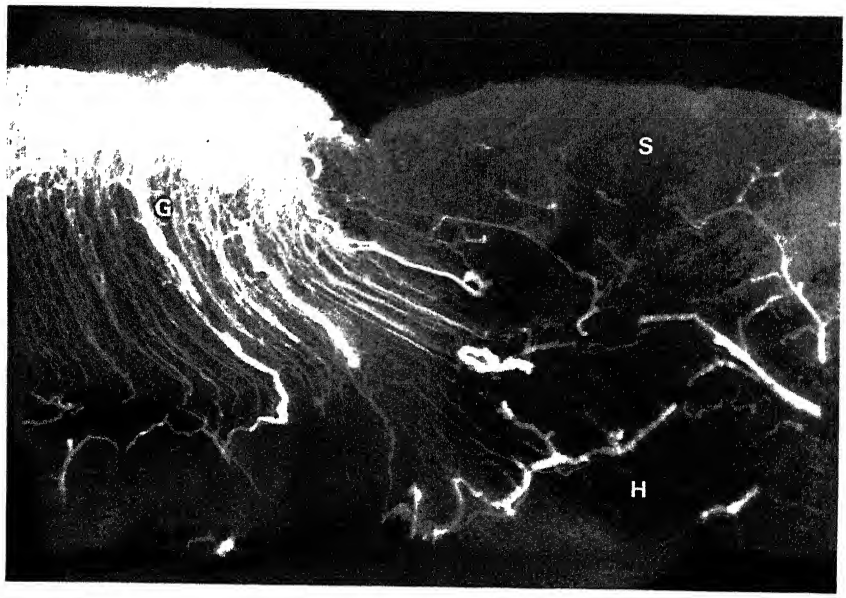
Scar tissue is functionally inferior to the uninjured dermis, and methods of improving the quality of repair by inducing scarless healing are being sought. In the fetus, cutaneous wounds heal with either little or no scarring, whereas postnatally scar tissue develops at the wound site. Whitby and Ferguson (1991a) in a comparison of fetal, neonatal and adult wound healing have found that the virtually scarless healing of fetal wounds is associated with the absence of transforming growth factor-$\beta$ (TGF-$\beta$) and basic fibroblast growth factor (b-FGF), and with the early deposition of fibronectin and tenascin. This has led to attempts to decrease scarring in postnatal wounds by administering neutralizing antibodies to TGF-$\beta$, with some success (Shah et al 1992). Recently the effects of the TGF-$\beta$ isoforms 1, 2 and 3 on scarring have been investigated (Levine et al 1993); TGF-$\beta$1 and TGF-$\beta$2 stimulate scarring whereas TGF-$\beta$3 appears to reduce it. The addition of mannose-6-phosphate which inhibits TGF-$\beta$ activation also reduces scarring and improves the quality of wound healing (Ferguson 1994). Manipulation of the growth factor profile at the wound site by neutralizing some factors and encouraging the synthesis of others may lead to improvement in the quality and rate of the healing response following injury.

The cellular changes involved in dermal repair can be accelerated by treatments which improve the microenvironment of the wound (Dyson et al 1988) and by application of electrotherapy modalities which increase the rate of ingress of macrophages (Dyson 1987, Dyson & Young 1986), possibly by temporarily modifying their membrane structure. The use of such techniques has considerable clinical and surgical significance.

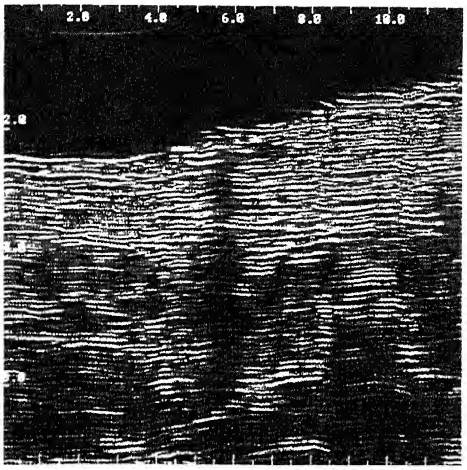
Until recently many of the reports of the clinical effectiveness of these and other techniques designed to improve the rate and quality of repair were based either on the subjective assessment of the progress of healing or on invasive techniques which inevitably interfered with the healing process by inflicting a further injury. The need for non-invasive, painless, objective methods of assessment has led to the development of high-resolution diagnostic ultrasound scanners in which changes in the ultrasonograms of the wound bed can be quantified by means of image analysis in which fractal signatures are produced of regions of interest (ROIs) of the ultrasonograms (Whiston et al 1992). A typical ultrasonic image of an incised cutaneous wound is shown in 5.77. Changes in fractal signatures obtained of ROIs of such images during the healing process are shown in 5.78. As healing progresses, the fractal signatures of the wound bed approach that of intact skin. Should the wound deteriorate, this process is reversed, the fractal signature becoming less similar to that of intact skin. This technique has been used to identify adverse changes at operation sites in renal transplant patients undergoing graft rejection (Calvin et al 1994). The technique can also be used to monitor the development of scar tissue at the wound site.

## EPIDERMAL REGENERATION

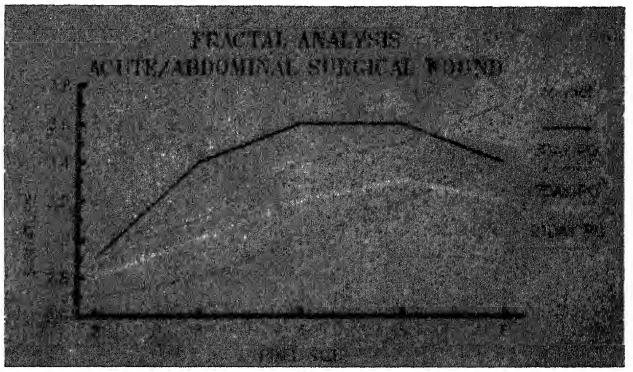
Changes in the epidermis leading to re-epithelialization begin within a few hours of the formation of a cutaneous wound. Intact keratinocytes at the free edge of the cut epidermis begin to migrate across the defect (Winter 1962). Migration is made possible by a change in gene expression of the cells, involving temporary dissolution of hemidesmosomes and desmosomes (p. 382), freeing the cells to move, and the formation of peripherally located actin filaments enabling them to do so (Gabbiani et al 1978). They also acquire a unique phenotype, termed the phenotype of regenerative maturation (Mansbridge & Knapp 1987), possibly as a result of exposure to low extracellular calcium concentrations (Hennings et al 1980; Clark




5.76 Microangiograph of a transverse section of granulation tissue (G) showing its invasion by new blood vessels; adjacent intact skin (S), and hypodermis (H) are also visible. The specimen was perfused with barium sulphate and gelatin 10 days after the production of a full-thickness excised lesion in porcine skin. The majority of the regenerating vessels lie at right angles to the surface and are linked by a superficially located capillary plexus. Magnification × 130. (Provided by S Young, Department of Anatomy, UMDS, Guy's Campus, London.)




5.77 High resolution ultrasonogram of skin and subcutaneous tissue containing an incised wound identifiable by reduced echogenicity. (Provided by S Young, Department of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)



5.78 Fractal signatures obtained from high resolution ultrasonograms of an incised cutaneous wound during the inflammatory (3-day PO), proliferative (7-day PO) and remodelling (21-day PO) phases of healing, together with the signature obtained from the skin prior to injury. As healing progresses the fractal signatures of the ultrasonograms of the injured skin approach that of intact skin. PO = postoperative. (Provided by S Young, Department of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)

1990). According to the 'leap-frog' hypothesis of epidermal regeneration (Winter 1962, 1964), cells superficial to the stratum basale at the edges of the wound elongate laterally and crawl over each other until they make contact with the wound bed; they then cease to move and begin to divide, producing a new supply of cells, some of which add to the thickness of the regenerating epidermis. Meanwhile other cells migrate over the first cells, reach the wound bed, divide and repeat the process in 'leap-frog' fashion until prevented from doing so by contact inhibition. According to this hypothesis no single keratinocyte moves more than about four or five cell diameters (approximately 40 μm) from its original position during epidermal regeneration. Within 48 hours of injury the basal keratinocytes of the new epidermis begin to divide, generating more cells capable of migration (Hell & Cruickshank 1963). The stimulus for epidermal proliferation after injury is still unknown. It may be the removal of an inhibitory chalone (Bullough & Laurence 1961) and/or the release of epidermal growth factors (Cohen 1965).

In shallow, partial thickness wounds of thin skin, each cut hair follicle acts as a source of reparative epidermal stem cells. These cells can be recognized by their ability to form a characteristic stem cell keratin (Type 19). After migration and division, they produce cells which manufacture other keratins (Types 9 and 16) which allow the cells to remain sufficiently flexible to migrate over the wound bed. Later products of keratinocyte division produce more rigid keratins (Types 1 and 10), typical of mature epidermis.

If the injury is sufficient to disrupt the basement membrane, the keratinocytes migrate over a temporary matrix of fibronectin, fibrin and Type V collagen (Clark et al 1982; Repesh et al 1982; Donaldson & Mahan 1983). Keratinocytes have been shown to secrete fibronectin in vitro (Kariniemi et al 1982; O'Keefe et al 1984; Kubo et al 1984) so it is possible that they may produce at least part of the matrix over which they migrate. TGF-$\beta$ encourages epithelial cell migration (Yang & Moses 1990) and stimulates fibronectin deposition (Nickoloff et al 1988). Once migration ceases, the temporary matrix is replaced by basement membrane.

Regeneration of the basement membrane zone occurs in sequential stages. Bullous pemphigoid antigen (p. 397) is always present between the basal plasma membrane of the migrating cells and the temporary matrix and can thus be considered to be the first part of the basement membrane to regenerate (Stanley et al 1981; Clark et al 1982). Once the epidermal cells cease to migrate, first Type IV collagen and then laminin become incorporated in the regenerating basal lamina (Clark et al 1982).

If the migrating keratinocytes make contact with small foreign particles of approximately 1 μm in size, the cells may remove them by phagocytosis (Odland & Ross 1968), possibly after opsonization by fibronectin (Takashima & Grinnell 1984). The keratinocytes migrate deep to any larger particles and dead tissues which lie in their path. Secretion of plasminogen activators (Isseroff et al 1982), collagenases and neutral proteases (Donoff et al 1971) by the keratinocytes may help to clear the way for their migration.

Once re-epithelialization is complete, the keratinocytes revert to their original phenotype (Clark 1985).

In humans and other mammals the breasts form a secondary sexual feature of females and are the source of nutrition for the neonate, although they are also present in a rudimentary form in males. Developmentally they are derived from modified sweat glands (**5.79**). In females, major growth and differentiation of breast tissues occurs after puberty to give rise to a complex structure, predominantly composed (in the non-lactating breast) of adipose tissue surrounding epithelial secretory tissue arranged in 15–20 lobes, each leading to a lactiferous duct which converges with the others upon the nipple. Connective tissue, blood vessels, lymphatics and nerves also contribute to breast structure. A specialized area of skin, the *areola*, surrounds the base of the nipple. The breasts are the site of malignant change in as many as one in ten women, and the biology of their tissues is at present the focus of much clinical research. For reviews of normal breast structure, see: Cowie (1974), Pitelka (1983), Ellis et al (1993), and Fawcett (1994).

In young adult females, each breast is a rounded eminence lying within the superficial fascia, chiefly anterior to the upper thorax but spreading laterally to a variable extent (5.79). Breast shape and size depend upon genetic, racial and dietary factors, together with age, parity and menopausal status of the individual, being hemispherical, conical, variably pendulous, piriform or thin and flattened. In the adult female the base of the breast (its attached surface) extends vertically from the second or third to the sixth rib, and in the transverse plane, from the sternal edge, medially, almost to the midaxillary line laterally. The superolateral quadrant is prolonged towards the axilla along the inferolateral edge of pectoralis major, from which it projects a little, and may extend through the deep fascia up to the apex of the axilla (the axillary tail of Spence).

The breast lies upon the deep pectoral fascia, which in turn overlies pectoralis major and serratus anterior, and below, obliquus externus abdominis and its aponeurosis as that forms the anterior wall of the sheath of rectus abdominis. Between the breast and the deep fascia is loose connective tissue in the retromammary (submammary) 'space', which allows the breast some degree of movement on the deep pectoral fascia. (Advanced mammary carcinoma may, by invasion, fix the breast to pectoralis major.) Occasionally, small projections of glandular tissue may pass through the deep fascia into the underlying muscle in normal subjects.

Before describing the general organization of the breast, the structure of the nipple and areola will be considered.

## NIPPLE (MAMMARY PAPILLA)

The nipple (5.79, 80) projects centrally from the anterior aspect; its shape varies from conical to flattened, depending on nervous, hormonal, developmental and other factors. Its level in the thorax varies widely but is at the fourth intercostal space in most young women, and in the nulliparous it is pink or light brown or darker, depending on the general melanization of the body. It is covered by hairless skin; the epidermis has a deeply folded base interdigitating with dermal papillae, and scattered sebaceous glands open on to its surface. Melanocytes are quite numerous, giving the skin of the nipple a darker hue. Internally the nipple is composed mostly of collagenous dense connective tissue with numerous elastic fibres which also spread beneath the areola, wrinkling the overlying skin. Smooth muscle cells are also present in and just deep to the nipple, disposed in a predominantly circular direction and radiating out from its base into the surrounding breast; their contraction, induced by cold or tactile (e.g. in suckling), or emotional stimuli causes erection of the nipple and wrinkling of the surrounding areola. The lactiferous ducts traverse the nipple, their 15–20 minute orifices opening on to its wrinkled tip. Near its opening at the nipple each of these ducts is slightly expanded as a *lactiferous sinus* in the lactating breast by the presence of milk. Occasionally the nipple may not evert during prenatal development (p. 296), remaining permanently retracted and so causing difficulty in suckling.

## AREOLA

The areola is a discoidal area of skin which encircles the base of the nipple (5.79, 80); its colour also varies from pink to dark brown depending on parity and race. Darkening of the nipple and areola occurs during the second month of pregnancy, and although it becomes a little paler after parturition, the change of hue is permanent. The nipple and especially the areola contains many sebaceous glands much enlarged in pregnancy and lactation as subcutaneous 'tubercles', whose oily secretion is a protective lubricant during lactation. Other glands (*areolar glands of Montgomery*) are intermediate in structure between lactiferous and sweat glands; when visible to the naked eye they are creamy in colour. At the perimeter of the areola are large sudorific and sebaceous glands, the latter not accompanied by hairs. There is no adipose tissue immediately beneath the skin of the areola and papilla.

## INTERNAL ORGANIZATION OF THE BREAST

The breast (5.79, 81–88) contains:

- epithelial glandular tissue of the tubulo-alveolar type
- fibrous connective tissue (stroma) surrounding the glandular tissue
- interlobar adipose tissue (Cowie 1974).

**Glandular tissue.** This consists of branching *ducts* and terminal secretory *lobules* (5.81–85). The ducts converge on to the 15–20 larger lactiferous ducts which open on to the apex of the nipple. Each lactiferous duct is therefore connected to a tree-like system of ducts and lobules, enclosed and intermingled with connective tissue stroma, collectively forming a *lobe* of the mammary gland; the number of lobes is, therefore, the same as the number of lactiferous ducts. Although the lobes are usually depicted as discrete anatomical territories within the breast, they grow into one another around their edges so that they do not appear as distinct entities during surgery.

*Lobules* consist of the portions of the glands that are secretory (or potentially so). Their structure varies according to hormonal status (see below), but in the mature breast each lobule consists of several blind-ending branches or expansions, the *alveoli* (*acini*), converging on an *alveolar duct*, and these are the sites of milk secretion.

Breast cancers arise at the junction of the lobules and ducts, and as they increase in size they lead to fibrous tissue formation so that they are hard and irregular.

**Stroma of the breast.** The connective tissue *stroma* penetrates between and encloses the lobules, where it has a loose texture, allowing the rapid expansion of secretory tissue during pregnancy (5.79, 84). Fibrous condensations of stromal tissue extend from the ducts to the dermis, and these are often well developed in the upper part of the breast as the *suspensory ligaments (of Astley Cooper)*, which assist in the support of the breast tissue. Pathologically, these may be contracted by fibrosis in carcinoma, causing retraction or pitting of the overlying skin. Elsewhere in the normal breast, fibrous tissue surrounding the glandular components extends to the skin and nipple, assisting in the mechanical coherence of the gland.

**Adipose tissue.** Highly variable in amount, this is typically present in the interlobar stroma, and not amongst the lobules.

## BREAST DEVELOPMENT

### Prenatal development

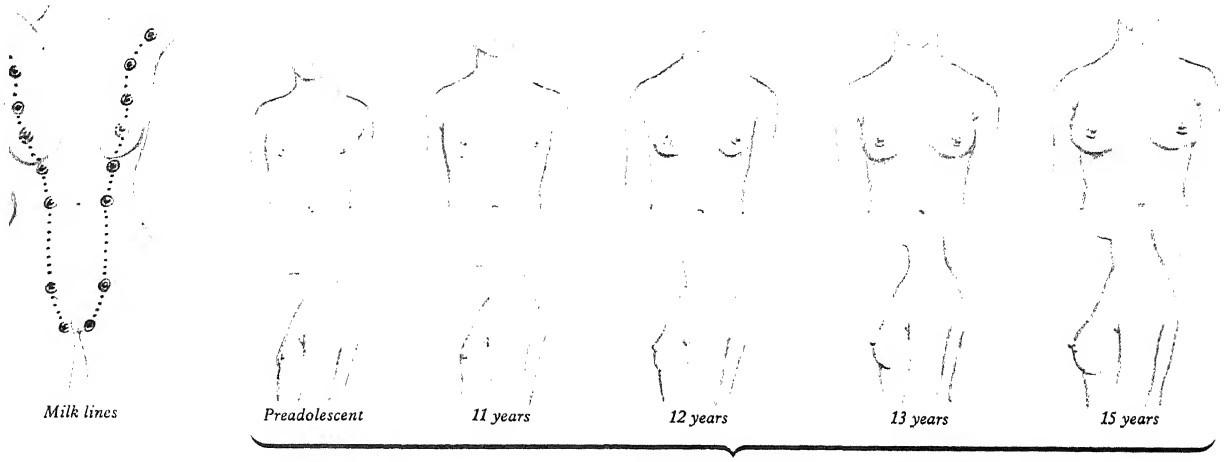
Prenatal development is similar in both sexes, with the epithelial mammary bud appearing at a gestational age of 35 days; by day 37 this has become a mammary line extending from the axilla through to the inguinal region (see also 5.79). Usually invagination of the thoracic mammary bud into the mesenchyme occurs by day 49, with involution of the remaining mammary line, although accessory breast tissue may be present in adults anywhere along the milk line (polythelia), usually in the thoracic region (90%) but also occasionally in the axillary (5%) or abdominal (5%). Nipple formation begins at day 56 and primitive ducts (mammary sprouts) develop at 84 days with canalization occurring at about the 150th day. In either sex, there may be no breast development (amastia), or alternatively there may be nipple development but no breast tissue (amazia). Rarely, the nipple may not develop (athelia) although this occurs more commonly in accessory breast tissue. At birth the combination of fetal prolactin and maternal oestrogen may give rise to transient hyperplasia and secretion of 'witch's milk'. (A fuller account of prenatal development is given on p. 296.)

### Postnatal development

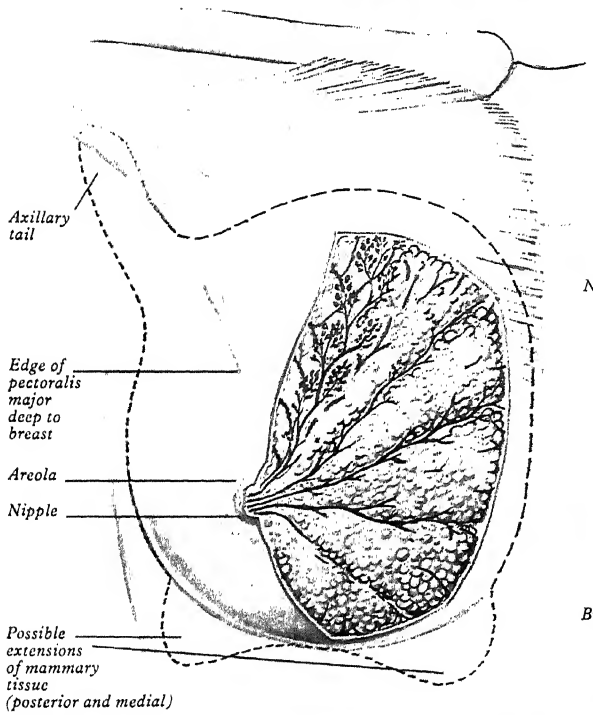
Lobule formation occurs (exclusively in females) after puberty (5.79), when there is branching of ducts and development of lobules from terminal ducts. Externally recognizable breast development (thelarche) from puberty onwards can be divided into five separate phases. In *phase I* there is elevation of the nipple. In *phase II* glandular subareolar tissue is present with both nipple and breast projecting from the chest wall as a single mass. *Phase III* encompasses increase in diameter and pigmentation of the areola, with proliferation of palpable breast tissue. During *phase IV* there is further pigmentation and enlargement in the areola so that the nipple and areola form a secondary mass anterior to the main part of the breast. Finally, in *phase V* there is development of a smooth contour to the

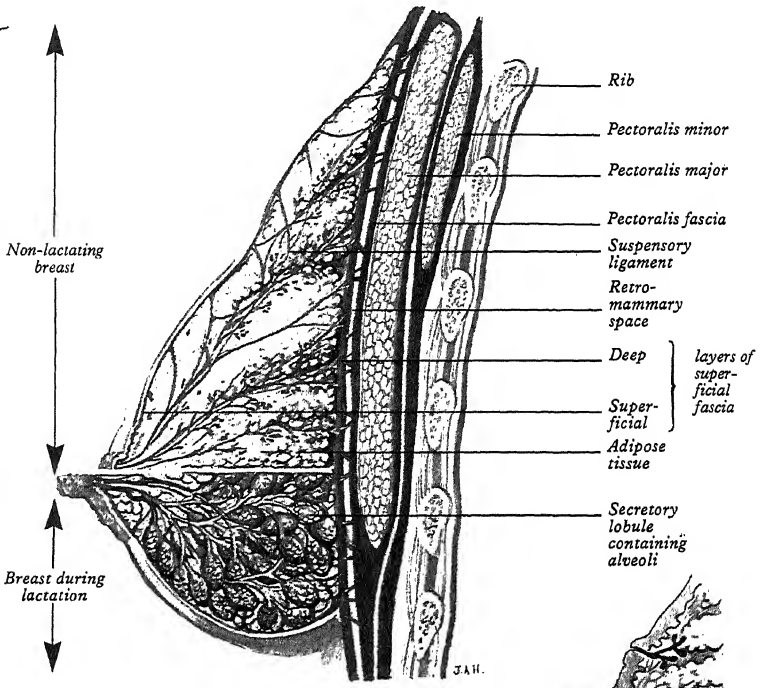


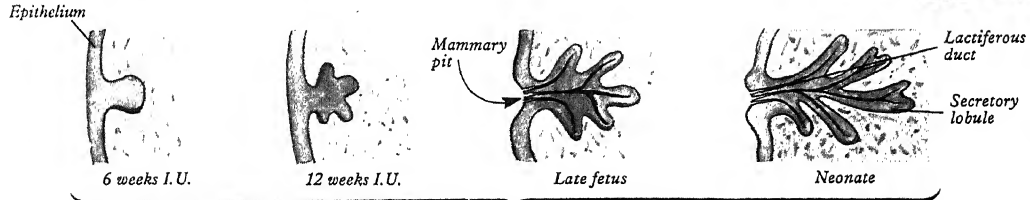
5.79 (*opposite*) Postnatal development and structure of the female breast.



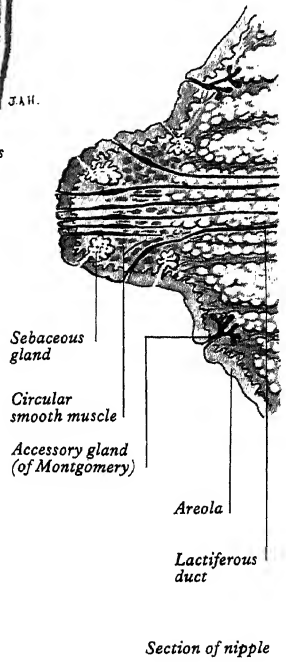

*Maturation of the breast*



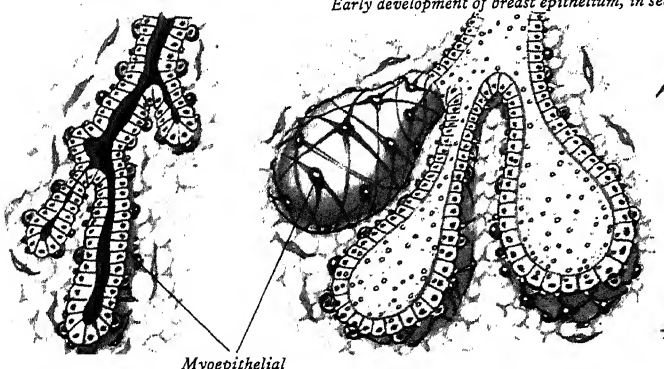




*Early development of breast epithelium, in section*

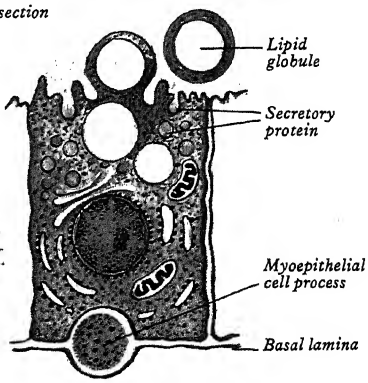



*Section of nipple*

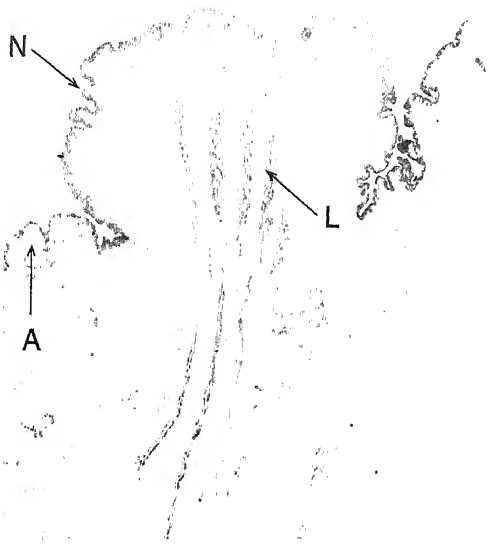



*HISTOLOGY:*
*Non-lactating (Nulliparous)*

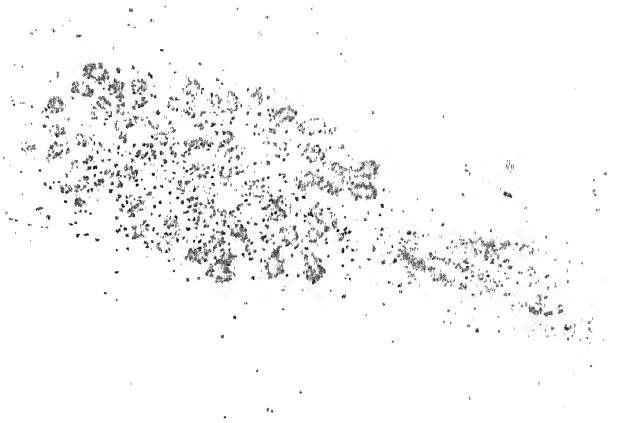
*Lactating*




*Ultrastructure of secretory cell*




5.80 Vertical section through female nipple, showing corrugated epithelium over the nipple surface (N), the surrounding areola (A), and lactiferous ducts (L). Haematoxylin and eosin. Magnification ×6. (Photography by Sarah Smith, Department of Anatomy and Cell Biology, UMDS, Guy's Campus, London.)



5.81 Normal non-lactating lobule with terminal duct, human breast. The duct can be seen leading to the lobule which is composed of multiple, small acini set within a loose intralobular stroma. The denser, interlobular stroma can be seen surrounding the lobule and duct. A small amount of adipose tissue is also present top right. Haematoxylin and eosin. Magnification × 450. (Provided by Dr Rosemary Millis, Consultant Pathologist, Guy's Hospital, London.)

breast. For further details of breast development, see page 296; for reviews: see Knight and Peaker (1982) and Anbazhagan et al (1991).

### Pregnancy

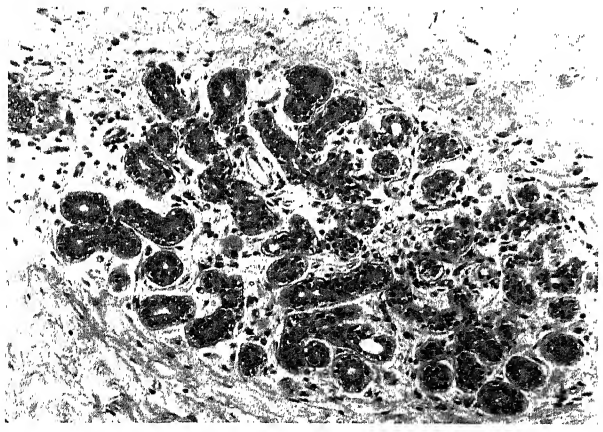
Changes during this period are associated with further duct and lobule proliferation and epithelial growth, consisting mainly of an increase in the number of alveoli per lobule (5.84). This is completed by the sixth month of pregnancy after which the breast expands further with the increase in blood flow and secretion of colostrum (see below). Total weight gain of each breast during pregnancy is about 400 g. True lactation starts within 1–4 days after parturition and may continue for as long as $3\frac{1}{2}$ years if frequent suckling is maintained. When lactation ceases there is a progressive atrophy of the lobules and ducts, with fatty replacement of breast tissue.

Changes also occur during the menstrual cycle, with an increase in size during midcycle, mainly due to a transient increase in blood flow, with consequent greater hydration of the stromal tissue; minor changes have been reported in epithelial structure too (see below), especially during the second half (luteal phase) of the cycle. With increasing age various changes take place in the proportions of the different components of the breast: after the menopause there is involution of the glandular tissue which may be replaced with adipose tissue, or the breast may gradually decrease in volume, and many other alterations take place in the mechanical properties, for example elasticity of the connective tissue supporting the breast.

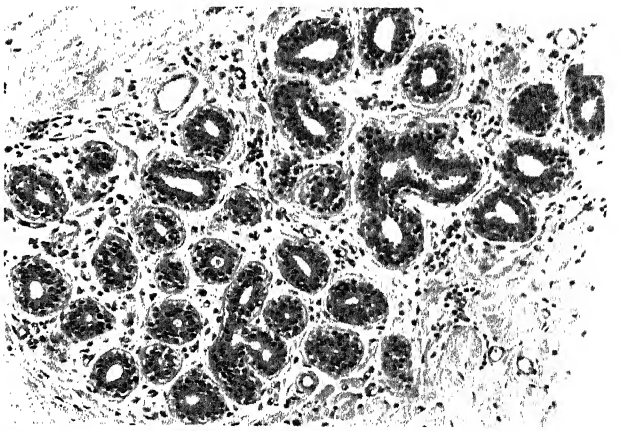
## DETAILED MICROSTRUCTURE OF THE BREAST

### Before pregnancy

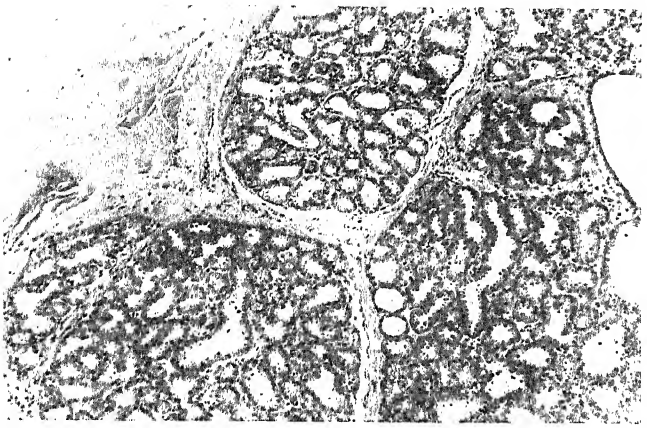
As already noted, mammary structure varies with age, time in the menstrual cycle, pregnancy and lactation. In the neonate there are lactiferous ducts but no alveoli, and until puberty little branching of the ducts occurs, the slight mammary enlargement being due to the growth of fibrous stroma and fat. After puberty, the ducts, stimulated



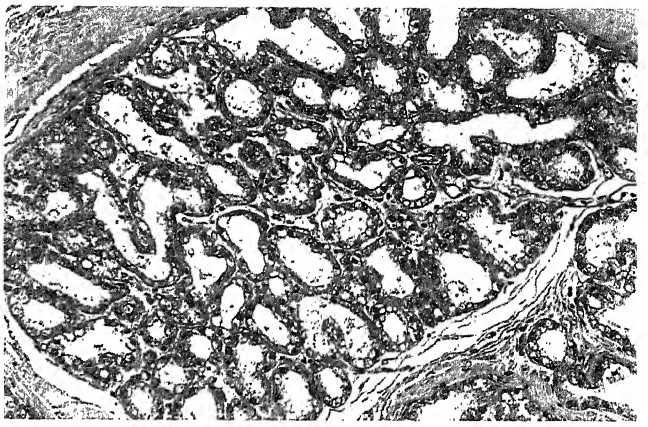
5.82 Normal non-lactating terminal duct lobular unit. The terminal duct can just be seen to the left of the picture. The lobule is composed of numerous acini. Haematoxylin and eosin. Magnification ×450. (Provided by Dr Rosemary Millis, Consultant Pathologist, Guy's Hospital, London.)



5.83 High power view of a non-lactating lobule. The two cell type lining of the acini can be clearly seen with the inner luminal epithelium and outer myoepithelial layer. The eosinophilic basement membrane can also be discerned. The loose intralobular stroma contrasts with the denser, interlobular stroma surrounding the lobule. Haematoxylin and eosin. Magnification ×800. (Provided by Dr Rosemary Millis, Consultant Pathologist, Guy's Hospital, London.)



5.84 Lactating breast. The lobules are greatly expanded. Many more acini are present lined by cells showing evidence of secretory activity. The intralobular stroma has largely been displaced. Haematoxylin and eosin. Magnification ×450. (Provided by Dr Rosemary Millis, Consultant Pathologist, Guy's Hospital, London.)



5.85 High power view of lactating breast. The enlarged lobule, composed of multiple distended acini, can be seen. The vacuolated cytoplasm of the secretory epithelium is clearly visible. Myoepithelium is difficult to see. Haematoxylin and eosin. Magnification ×450. (Provided by Dr Rosemary Millis, Consultant Pathologist, Guy's Hospital, London.)

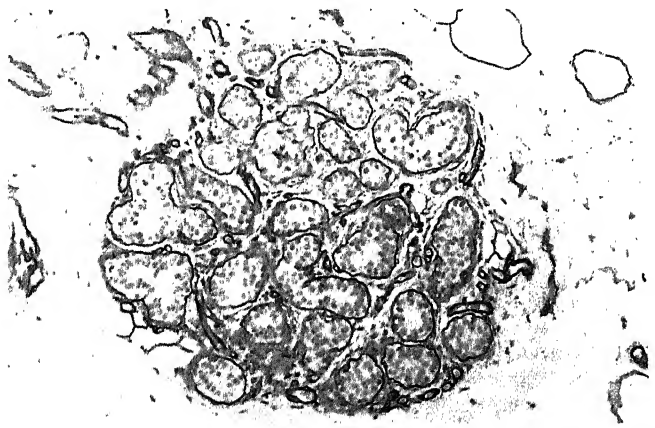
by ovarian oestrogens, develop branches whose ends form solid, spheroidal masses of granular polyhedral cells, which are potential alveoli (Stirling & Chandler 1977). In the resting state the glandular epithelium is separated from the vascular stroma by a thin avascular zone of fibroblasts (**5.83**). This 'epitheliostromal junction' may control the passage of materials to the secretory cells (Ozzello 1974) and have other important controlling influences on breast biology, mediating the actions of hormones on growth, cell division and secretion.

**Ductal system.** For most of their lengths, the ducts are lined by columnar epithelium (**5.81–83**); in the larger ducts these are two cells thick, but in the smaller ones only a single layer of columnar (*luminal*) cells is present. The bases of these are in close contact with numerous *myoepithelial* (*basal*) cells which invaginate their bases (**5.87**), and are so frequent that they form a distinct layer surrounding the ducts and alveoli, giving the epithelium a bilaminar appearance.

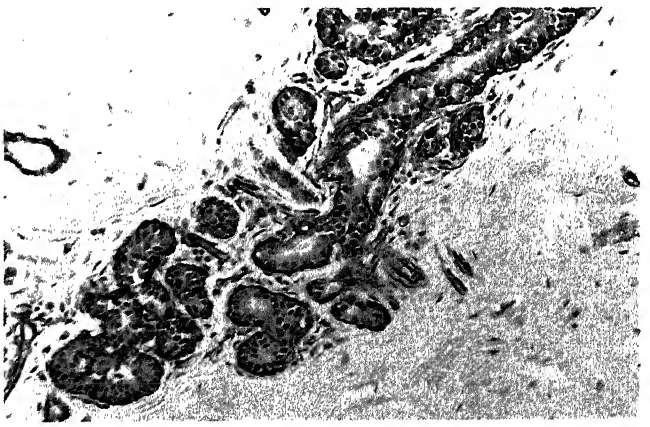
Close to the openings of the lactiferous ducts on to the nipple surface, their stratified cuboidal lining gives way to keratinized stratified squamous epithelium continuous with the epidermis; shed squames may sometimes block the duct apertures in the non-pregnant breast. External to the epithelial lining is a *basement membrane* (**5.86**) composed of a thin *basal lamina* and a more extensive external *reticular region* blending with the stroma. The structure of the ductal complex varies with development and hormonal status, postpubertal maturation, the menstrual cycle, pregnancy and age-related regression. These factors have considerable effect on the microscopic structure of the deeper parts of the ducts (see below).

## During the menstrual cycle

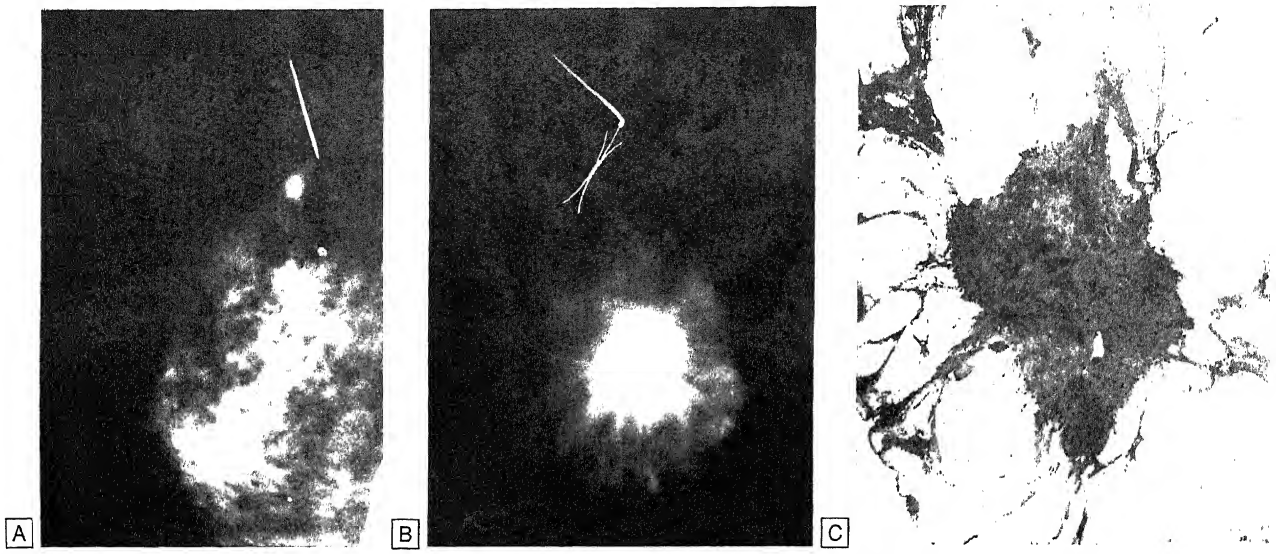
Demonstrable changes occur to the breast tissues in the menstrual cycle (Fanger & Ree 1974; Ferguson et al 1992); in the follicular phase (days 3–14) the stroma becomes less dense and various changes take place in the ducts, including the expansion of their lumen, with occasional mitoses but no secretion. In the luteal phase (days 15–28) there is a progressive increase in stromal density; the ducts have an open lumen containing secretion, with flattening of epithelial cells. Cell proliferation, as measured by $^{3}$[H]-thymidine labelling, is maximal on day 26. Thereafter, the ductal system undergoes reduction, with epithelial cell apoptosis greatest on day 28 of the cycle. These activities have considerable clinical significance in terms of the most appropriate timing for surgery related to carcinoma of the breast (see Fentiman 1993).



5.86 Normal non-lactating lobule stained by immunohistochemistry for collagen IV to demonstrate basement membrane. The acini of the lobule are clearly seen. Each lobule is surrounded by a well defined basement membrane. Basement membrane material can also be seen surrounding vessels and fat cells. (Immunoperoxidase method for collagen IV). Magnification ×450. (Provided by Dr Rosemary Millis, Consultant Pathologist, Guy's Hospital, London.)



5.87 Normal lobule. Stained by immunohistochemistry for actin to demonstrate myoepithelial cells. The terminal duct lobular unit can be seen. The myoepithelial cells have stained positively with the antibody to actin. A limiting ring of myoepithelial cells is seen around the ducts and acini. Staining is also demonstrated in vessel walls. (Immunohistochemistry for actin.) Magnification ×450. (Provided by Dr Rosemary Millis, Consultant Pathologist, Guy's Hospital, London.)




**5.88** A mammogram (A), specimen radiograph (B) and histological section (C) of an infiltrating carcinoma.

A. Mammogram of the breast with a rounded density in the upper half which has an irregular, spiculated margin. Adjacent to the density is a needle which has been inserted to localize the lesion for the surgeon prior to operation. Elsewhere in the breast foci of calcification are seen. These are large, single particles, with a smooth outline and are typical of benign calcifications.

B. Radiograph of the surgical specimen after it has been removed from the patient. This confirms that the mammographic abnormality has been removed. Note the adjacent marker needle. The irregular margin of the rounded density can be more clearly seen in the specimen radiograph.

C. Low power photomicrograph of the histological section showing the carcinoma in the centre with surrounding adipose tissue. The rather rounded outline of the tumour can be seen with an irregular margin which corresponds to the density seen on the radiograph. (Provided by Dr Rosemary Millis, Consultant Pathologist, Guy's Hospital, London.)

Immunolabelling shows that various antigens associated with the basal lamina of the ducts and alveoli, including laminin, Type IV collagen and fibronectin, undergo major changes during the cycle, whereas other stromal components are relatively unaffected (Ferguson et al 1992). This finding adds weight to the concept that the basal lamina is involved in the trophic control of secretory tissue behaviour.

In addition to these alterations there are changes in blood flow, which are greatest at midcycle, with a consequent increase in the water content of the stroma at that time.

## In pregnancy and during suckling

As the output of placental oestrogen and progesterone rises during pregnancy, the ducts increase in the number and lengths of their branches; the secretory alveoli proliferate, and with the synthesis and secretion of milk the alveoli expand as their cells and lumen fill (**5.84, 85**). The myoepithelial cells, which are initially spindle-shaped, become highly branched stellate cells, especially around the alveoli. In the stroma there is a concomitant reduction in adipose tissue, but the numbers of lymphocytes including plasma cells, and of eosinophils increase greatly; blood flow through the breast also increases. The secretory activity in alveolar cells rises progressively in the latter half of pregnancy. Their product in late pregnancy and for a few days after parturition is different from the later milk and is known as *colostrum*; it contains many cytoplasmic fat globules and colostral corpuscles, which are a combination of epithelial cell membranes and macrophages, and is rich in immunoglobulins, conferring a measure of passive immunity to the neonatal alimentary tract (see also below); it also has laxative properties. True milk secretion begins a few days after parturition due to a reduction of circulating oestrogen and progesterone, a change which appears to stimulate production of prolactin by the anterior hypophysis (Wolstenholme & Knight 1972 and p. 1883). Milk distends the alveoli, at first lined by a single layer of granular, short columnar cells with stellate myoepitheliocytes near the basement membrane; the cells flatten as secretion increases. The columnar cells show accumulation of fat droplets which then undergo apocrine secretion into the lumen. After the onset of lactation there is a gradual reduction in the numbers of lymphocytes and eosinophils in the stroma, although plasma cells continue to synthesize IgA for secretion into the milk.

## Postlactational breast

When lactation ceases the secretory tissue undergoes some involution, but the ducts and alveoli never return completely to the pre-pregnant state. Two major processes are responsible for the regression of the alveolar-ductal system, a reduction in epithelial cell size, and a reduction in their numbers. Size reduction appears to involve the formation of lysosomal autophagic vacuoles within the cells, reducing the numbers of their organelles and cytoplasmic volume. The loss of cells may be by apoptosis, some dead cells being shed into the lumen. At the same time, macrophages invade the stroma, and some of them also enter the alveolar/ductal lumen; in both of these sites they phagocytose dead epithelial cells, and also appear to modify or destroy the basal lamina of the epithelium, with consequent loss of epithelial cell activity which apparently depends on the integrity of this structure. Eventually, the numbers of lymphocytes and macrophages become reduced and gradually the breast tissue reverts to the resting state. If another pregnancy occurs the resting glandular tissue is reactivated, and the process outlined above recurs. Up to the age of about 50 increasing amounts of elastic tissue tend to be laid down around vessels and ducts (elastosis), and also in the stroma, although elastosis does not typically continue thereafter except in pathological changes (Martinez-Hernandez et al 1977; Farahmand & Cowan 1991).

## Postmenopausal changes

After the menopause there is progressive atrophy of lobules and ducts, with fatty replacement of breast tissue, although a few ducts may remain (Ozzello 1974); the stroma becomes much less cellular and collagenous fibres decrease; the amount of adipose tissue varies widely between individuals, but the breast may return to a condition similar to the pre-pubertal state.

## Cellular structure

As outlined above, the cells of the breast include:

- *epithelial cells*: alveolar, duct-lining and myoepithelial cells
- *connective tissue cells of the stroma*: fibroblasts, adipocytes, mast cells, macrophages, lymphocytes, neutrophils and eosinophils.

In addition there are cells associated with vessels and nerves.

**Alveolar cells** (5.79, 85, 88). As already stated, these display wide ultrastructural differences depending on the physiological state of the breast. In the resting state they are cuboidal. The apical surfaces are rich in microvilli and adjacent cells are joined around their apical ends by junctional complexes including tight junctions, adhering junctions and desmosomes; gap junctions are also present between these cells (Pitelka et al 1973). In lactation they increase in height initially to a columnar form, but as the alveoli distend with milk, stretching their epithelial linings, they may become cuboidal again. In the *secretory phase* when viewed by light microscopy, their apical cytoplasm is eosinophilic and vacuolated, and their apical surfaces often bulge into the alveolar lumen, distended by relatively huge secretory vacuoles; basally, their cytoplasm is basophilic. Ultrastructurally, the basal region possesses abundant granular endoplasmic reticulum, mitochondria, lysosomes and free ribosomes. Apical to the basally-situated nucleus are a Golgi complex and large secretory vacuoles of two types, one proteinaceous and the other lipidic. *Protein vacuoles* contain multiple granules of micellar *casein* and other lactic proteins formed in the granular endoplasmic reticulum and passed to the Golgi apparatus to form larger vacuoles; these vacuoles are passed to the apical surface where they release their contents by membrane fusion (merocrine secretion). *Lipid vacuoles*, on the other hand, are formed directly in the apical cytoplasm as smaller lipid droplets which fuse with each other to create large 'milk vacuoles' up to 10 μm across, frequently protruding from the cell's surface. These are released as intact lipid droplets with a thin surround of apical plasma membrane and adjacent cytoplasm (Linzell & Peaker 1971; Saacke & Heald 1974; Hollmann 1974; Tobon & Salazar 1975; Pitelka & Hamamoto 1977; Hartmann 1991). This secretory process may be considered to be apocrine, since actual cytoplasm is lost with the secretion, although only minimally. In pregnant rats, other types of protein granule are also synthesized as precursors of normal granules; these may appear in colostrum (Murad 1970). Alveolar cells also take up IgA from adjacent plasma cells by endocytosis, and secrete it apically by a separate, merocrine mechanism.

**Duct-lining (luminal) cells.** The detailed structure of these varies with the diameter of the duct, but most of them are columnar to cuboidal in shape (5.81). They have relatively few organelles, and their nuclei are elliptical and euchromatic with a rim of condensed chromatin. Like other epithelial cells of the mammary gland the ductal cells are capable of cell division when hormonally stimulated, although it is not clear whether a distinctive stem cell population is responsible for this activity, or if all ductal cells can act in this capacity.

**Myoepithelial cells** (5.79, 87). These are similar to this type of cell in other glands (see pp. 71, 1695). In the mature mammary gland they are closely associated with the bases of alveolar and ductal cells and the long radiating, branched processes of adjacent myoepithelial cells intermesh to form a basket-like network around the alveoli and ducts, interposed between the basement membrane and the luminal cells. Internally they contain actin (5.87) and myosin filaments. Immunohistochemistry shows that they contain antigenic markers for epithelial features such as cytokeratins and also for smooth muscle (e.g. desmoplakins); a subpopulation is also positive for glial fibrillary acidic protein (GFAP) (see Viale et al 1991; Lazard et al 1993). On suitable hormonal stimulation by oxytocin they contract to expel the secretions into the larger ducts in readiness for suckling.

**Stromal components.** The cells of the stroma resemble those of other connective tissues elsewhere in the body. However, there is much evidence that they interact closely with the epithelial cells, and are an essential part of the hormonal regulatory system which controls the activity of the secretory tissue. This has been demonstrated for a number of stromal cell types in co-culture (including the numerous adipocytes) which are necessary for the stimulation of mammary epithelial cell growth and differentiation (Blum et al 1987). *B-* and *T-lymphocytes* are present throughout the stroma, but are particularly numerous around the ducts and alveoli, and also between the epithelial cells themselves. These cells provide immune surveillance of the stroma and epithelium; mature B-cells (plasmacytes) around the secretory regions are the source of immunoglobulins secreted during lactation. *Macrophages* have a distribution similar to that of lymphocytes, being both stromal and intraepithelial. In addition, some macrophages enter the lumen of the ducts, where they can phagocytose the shed epithelial cells, as noted above. Macrophages are also present in colostral milk although their significance in this respect is uncertain. Macrophages are important in regressional changes following lactation, where they dispose of degenerating epithelial cells. They are also likely to be important sources of growth factors and other chemical agents affecting the biology of the breast tissue, as they do elsewhere in the body (see p. 78).

## LACTATION

The release of milk during suckling depends upon a combination of touch and negative pressure from the infant's lips on the nipple (Sala et al 1974). Stimulation of the abundant nerve terminals in the dermis of the nipple leads to oxytocin release, causing contraction of myoepithelial cells of the breast and the nipple's smooth muscle, increasing the pressure within the lactiferous ducts, and bringing about milk ejection. Cessation of suckling results in an increased intraluminal pressure which inhibits the secretory activity of the alveolar cells and subsequently the synthesis of milk.

Commonly lactation continues for 5 or 6 months after parturition, but then it progressively diminishes, according to demand, infants usually being weaned at about 9 months, although in some cultures suckling may be continued for over 3 years, during which the mother's ovulation is inhibited (see e.g. Thapa et al 1988). When lactation stops, the glandular tissue returns to the 'resting' condition, the remaining milk is absorbed and the alveoli shrink, many losing their lumina. However, due to hormonal and other disturbances, glandular tissue may fail to produce milk throughout pregnancy or secretion may cease within a few weeks of birth.

During lactation the volume of milk secretion by a mother is considerable, typically over 1100 ml/day (and nearly double this volume for twins). Amongst other nutrients, this creates a heavy demand for calcium which is obtained, as are other precursors of milk, from the circulation; interestingly, a hormone similar to parathyroid hormone has been shown to be secreted into the circulation from the lactating breast, assisting in the mobilization of calcium from storage sites in bones (Thiede & Rodan 1988).

### Milk

Milk is a complex fluid, composed in humans of about 88% water, 7% lactose, 4% fat, 1% protein and various ions, notably calcium, sodium, potassium, phosphate and chloride. Vitamins and antibodies, mainly of the IgA (secretory) class, are present, the latter being largely responsible for the sterility of milk during lactation (Jenness 1974). The proteins are chiefly caseins and lactalbumin; these, with lactose and several triglycerides, are synthesized from circulating precursors by enzymes. Colostral milk is markedly different, poor in nutrients with an ionic composition like blood plasma. Table 5.1 sets out the composition of human milk. For details of lactation and human mammary structure see the review by Vorherr (1979).

**Table 5.1 Major constituents of mature human milk**

| | | |
|---|---|---|
| **Protein** | total | 10.6 g/l |
| Casein | | |
| Lactalbumin | | |
| Albumin | | |
| Immunoglobulin | | |
| **Carbohydrate** | total | 78 g/l |
| Lactose | | 71 g/l |
| Oligosaccharide | | 6 g/l |
| Fucose | | 1 g/l |
| **Fats** | total | 45.4 g/l |
| **Water** | | 897 g/l |
| **Minerals** | | |
| Sodium | | 172 mg/l |
| Potassium | | 512 mg/l |
| Calcium | | 344 mg/l |
| Magnesium | | 35 mg/l |

### Vessels and nerves

**Arteries.** Supplying the female breasts are branches of the axillary artery, the internal thoracic artery, and some intercostal arteries, as follows:

- the *axillary artery* supplies blood to the breast via several branches: the supreme thoracic, the pectoral branches of the thoraco-acromial artery, the lateral thoracic and the subscapular artery;
- the *internal thoracic artery* gives perforating branches to the anteromedial part of the breast;
- the *second to fourth intercostal arteries* give perforating branches more laterally in the anterior thorax. The second perforating artery is usually the largest, supplying the upper region of the breast, and the nipple, areola and adjacent breast tissue (Bertelli & Valle Pereira 1994).

For further details, see page 1534).

**Veins.** Around the areola there is a circular venous plexus. From this and from the glandular tissue, blood drains in veins accompanying the arterial blood supply, i.e. to the axillary, internal thoracic and intercostal veins. Great individual variation may occur, and the axillary vein may be bifid. (See also p. 1592.)

**Lymph vessels.** The lymphatic drainage of the breast can be very variable (see Turner-Warwick 1959; also p. 1615). From the subareolar plexus (of Sappey) there are efferent vessels draining to the following:

- the contralateral breast
- the internal mammary lymph node chain, and thence via:
  1. the mediastinal lymph nodes to the para-aortic lymph nodes, bronchomediastinal trunks, thoracic duct and right thoracic duct
  2. inferiorly, the superior and inferior epigastric lymphatic routes to the groin
- the axillary lymph nodes, the predominant site of drainage from the breast. These number from 20–40; in the past these were named and grouped artificially as lower, central, subscapular, lateral and apical. Nowadays, a simpler nomenclature is generally adopted, based on the relation of the nodes to pectoralis minor. Those lying below pectoralis minor are the *low nodes* (level 1), those behind the muscle are the *middle group* (level 2), while the nodes between the upper border of pectoralis minor and the lower border of the clavicle are the *upper* or *apical nodes* (level 3). In addition, between pectoralis minor and major there may be one or two other nodes (*Rotter's nodes*). (See also p. 1613.)

**Nerves.** The nerve supply of the breast is derived from the anterior and lateral branches of the fourth to sixth intercostal nerves which carry sensory and sympathetic efferent fibres. The nipple supply is from the anterior branch of the lateral cutaneous ramus of T4; this forms an extensive nerve plexus within the nipple (see Miller & Kasahara 1959), its sensory fibres terminating close to the epithelium as free endings, Meissner corpuscles and Merkel disc endings (see p. 967). These are essential in signalling suckling to the central nervous system; however, secretory activities of the gland are largely controlled by ovarian and hypophyseal hormones rather than by efferent motor fibres.

## CLINICAL ASPECTS OF THE FEMALE BREAST

Breast cancer (**5.88A–C**) is a common disease, particularly in post-menopausal women (see Wellings 1980; Fentiman 1993). Breast lumps may be classified into those with clinical signs suggesting malignancy (hard and regular, skin-tethering, muscle fixation, skin infiltration or oedema (peau d'orange)), and those which are mobile and without sinister signs. Investigations include needle aspiration which will drain cycts, or in the case of a solid lump, obtain cells for cytological evaluation. Additional investigations include mammography and ultrasonography which can distinguish cysts from solid lumps. A common problem in young women is the fibroadenoma, an overgrowth of a lobule.

If a breast lump has to be removed, incision should be based whenever possible on Langer's lines (p. 381) for best cosmetic results, although for women with larger lumps which may be malignant, the incision should be compatible with a possible subsequent mastectomy. Most women with single breast cancers up to 4 cm in diameter are treated by breast conservation rather than mastectomy. This is a combination of surgery (tumour excision and axillary lymph node sampling or clearance) together with external radiotherapy. Patients with larger tumours are treated by modified radical mastectomy with clearance of the axilla and preservation of the nerves to serratus anterior, latissumus dorsi and the lateral and medial pectoral nerves. Failure to preserve the nerve to serratus anterior will result in winging of the scapula and reduced function of the shoulder.

Patients with blood-stained nipple discharge without a palpable lump are treated by duct excision (microdochectomy) carried out through a circumareolar incision. Blood-stained nipple discharge is caused by either an intraduct papilloma, or duct extasia, and only rarely (5% of cases) is it due to malignancy.

The papillary ducts are radially orientated and incisions should hence also be radial. An obstructed lactiferous duct may distend as a galactocele. Abscesses may occur between the septa in the glandular tissue, subcutaneously near the papilla or between gland and deep fascia anterior to the pectoralis major.

Supernumerary mammae (polymastia) or papillae (polythelia) occur in males and females, usually along a line extending from the axilla to the pubic region, the milk line (**5.79**).

The male breast remains rudimentary throughout life. It is formed of small ducts (without lobules or alveoli) and a little supporting fibro-adipose tissue (see Ellis et al 1993). Sometimes the 'ducts' are largely solid cellular cords. Slight temporary enlargement may occur at puberty. The areola is well developed, although limited in area, and the nipple is relatively small (for surface anatomy, see Irstam 1962). It is usually stated that the ducts do not extend beyond the areola, but a recent survey (Cochrane et al 1992) has shown that although this is generally true, the limits of the glandular tissue may extend well beyond this boundary (35% in a sample of 40 cadavers). The male mamma may hypertrophy after puberty (gynaecomastia), usually due to imbalance between oestrogenic and androgenic hormones. (See pp. 1883, 1884, 1895 and 1905 for endocrine influences.) Male breast cancers comprise approximately 1% of all mammary malignancies (Crichlow & Galt 1990), and may include tissue beyond the areolar boundary.

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